

## ANTI-CANCER EFFECT OF SAPONINS ISOLATED FROM *SOLANUM TRILOBATUM* LEAF EXTRACT AND INDUCTION OF APOPTOSIS IN HUMAN LARYNX CANCER CELL LINES

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### ABSTRACT

**Objectives** Pharmacological and chemical investigations of medicinal plants have provided a wide variety of natural compounds to possess significant cytotoxic as well as chemopreventive activity. *Solanum trilobatum*, a biomedicine and an anticancer drug have been used extensively as an indigenous drug in Indian system of traditional medicine, as an antioxidant rejuvenerator. Although some studies have demonstrated its antitumour activities on cancer cells *in vitro* and *in vivo*, the exact mechanism is not fully elucidated. Hence, the present study was designed to examine the *in vitro* cytotoxic activities of the saponin fraction isolated from *Solanum trilobatum* (SFST) on HEP-2 cell line.

**Methods** In this study the cytotoxic effect of the drug was confirmed by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Meanwhile, 4, 6-diamidino-2-phenylindole (DAPI) staining and agarose gel electrophoresis were performed for determining the induction of apoptosis.

**Key findings** The present results confirm a dose-dependent suppression of cell proliferation and the IC<sub>50</sub> value was found to be 1000µg/ml. At a dose of 1000µg/ml, marked morphological changes including cell shrinkage and condensation of chromosomes were observed. Agarose gel electrophoresis of DNA from HEP-2 cells treated with 1000µg/ml SFST for 24 hr showed marked DNA ladder pattern. In addition the involvement of free radicals was confirmed by increased superoxide production, decreased nitrate formation and depletion of glutathione in SFST-treated cells.

**Conclusions** The current work opens a new standpoint to validate *Solanum trilobatum* saponin as a candidate of anti-cancer drug for cancer therapy based on ethnopharmacological studies.

**Keywords:** *Solanum trilobaum*, HEP-2 cells, Apoptosis, cancer, Reactive Oxygen Species, Cytotoxicity, Nitric Oxide, Reduced glutathione, Saponin

### INTRODUCTION

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cells [1]. Laryngeal cancer is the second most common respiratory cancer after lung cancer and is relatively common cancer in men, but rarer in women. The augmentation of chemotherapeutic or chemopreventive means for laryngeal carcinoma is significant to reduce the mortality caused by this disease. The area of anticancer remedies gained massive attentions from scientists all over the world [2] with better indulgent of pathophysiology of the disease. Since cell homeostasis depends on the balance between proliferation and apoptosis, effective compounds inducing apoptosis appear to be a relevant strategy to suppress tumor growth [3]. Many chemotherapeutic drugs eliminate cancer cells by inducing, a genetically programmed form of cell death [4]. Plants and plant products both as extracts and derived compounds are known to be effective and versatile chemopreventive agents against various types of cancers [5, 6]. Identification of phytochemicals is found to be effective chemopreventive agents in the field of chemoprevention research [7].

Herbal remedies have been used for various treatments from the days of caveman. Traditional medicine has been widely used to heal cancer in about 75-80% of world population [8, 9, 10, & 11]. Increased demand for herbal products is the 'back-to-nature' global trend and the belief of the general public is that natural products are much safer without deleterious side effects. *Solanum trilobatum*, a medicinal treasure (rejuvenator), belongs to the family Solanaceae commonly found in Deccan peninsula in India. The extract of various parts of the plant is used in chronic bronchitis [12]. *Solanum trilobatum* is used in the treatment of tuberculosis and all kinds of lung disease [13]. Further, it is widely used to treat respiratory diseases in Indian traditional system of Medicine [13].

*Solanum trilobatum* possessed a broad spectrum of antibacterial, antifungal, free radical scavenging and antitumoural activities [15]. Informations have shown that *Solanum trilobatum* possessed an antitumor effect against chemically induced tumors [16, 17, & 18]. Studies

also revealed the chemopreventive effect of *Solanum trilobatum* against carbon tetra chloride and DEN (N-diethylnitrosamine) induced toxicity due to its antioxidant activity thus exerting and hepatoprotective property [19]. *Solanum trilobatum* extract showed the presence of compounds including solasodine, linoleic acid, palmitic acid, oleic acid and stearic acid [20]. Partially purified fraction of *Solanum trilobatum* contained sobatum, β-sitosterol, disogenin and β-solamarine responsible for many biological properties [18]. Hence the study was taken to establish the chemopreventive efficacy of the saponin fraction isolated from *Solanum trilobatum* (SFST) by evaluating cytotoxicity and apoptosis induction in the human larynx carcinoma (HEP-2) cell lines.

### MATERIALS AND METHODS

#### Chemicals

All reagents used in the study were of analytical grade. Open-column chromatography silica gel 60 as stationary phase, n-hexane, chloroform and methanol by gradient elution technique for sterols. TLC Silica gel F254 (Merck). HPLC Jasco binary system. Chloroform - methanol as a gradient mobile phase for saponin isolation. Stock solution of the saponin extract was dissolved in PBS containing 0.5% dimethyl sulfoxide (DMSO). The stock solutions were diluted with the medium to the desired concentration (the final concentration of DMSO on the medium was <0.01%, which had no detectable effect on cell growth). Eagle's minimal essential media (SMEM) and fetal bovine serum (FBS) were purchased from GIBCO. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), DAPI, proteinase K, phenylmethanesulfonyl fluoride (PMSF), rhodamine 123, and RNase A were purchased from Sigma Chemical Company (St. Louis, MO, USA).

#### Preparation of saponin fraction

Freshly collected leaves of *Solanum trilobatum* were cleaned, shade dried. These leaves were coarse powdered in a low speed blender and stored in an airtight container. The powdered sample was defatted by using petroleum ether 3 x1h at 40°C. After filtering the petroleum ether, the sample was extracted with methanol 3x1 h with mild heating. This methanolic extract was concentrated under

reduced pressure to obtain dark-colored residue (25 g). This residue was dissolved in water and then partitioned with n-butanol. n-butanol layer was separated and distilled off to yield crude saponin. This residue was used for the isolation of saponin compounds by column chromatography. TLC of methanol, butanol fractions was developed in suitable solvent system [CHCl<sub>3</sub>: Glacial acetic acid: MeOH: Water, 16:8:3:2 derivitised with Anisaldehyde -sulphuric acid

reagent] was give five separated spots (Figure a). HPLC studies of methanolic extract of *solanum trilobatum* extract by C18 with acetonitrile: water (3:7) at 1ml/min flow rate showed presence of four saponins (Figure b & c). The HPLC of isolated saponin was performed by same method which has given single peak at Retention Time of 2 min. This peak was also present in HPLC graph of methanolic extract.



Fig. a: TLC of isolated saponin

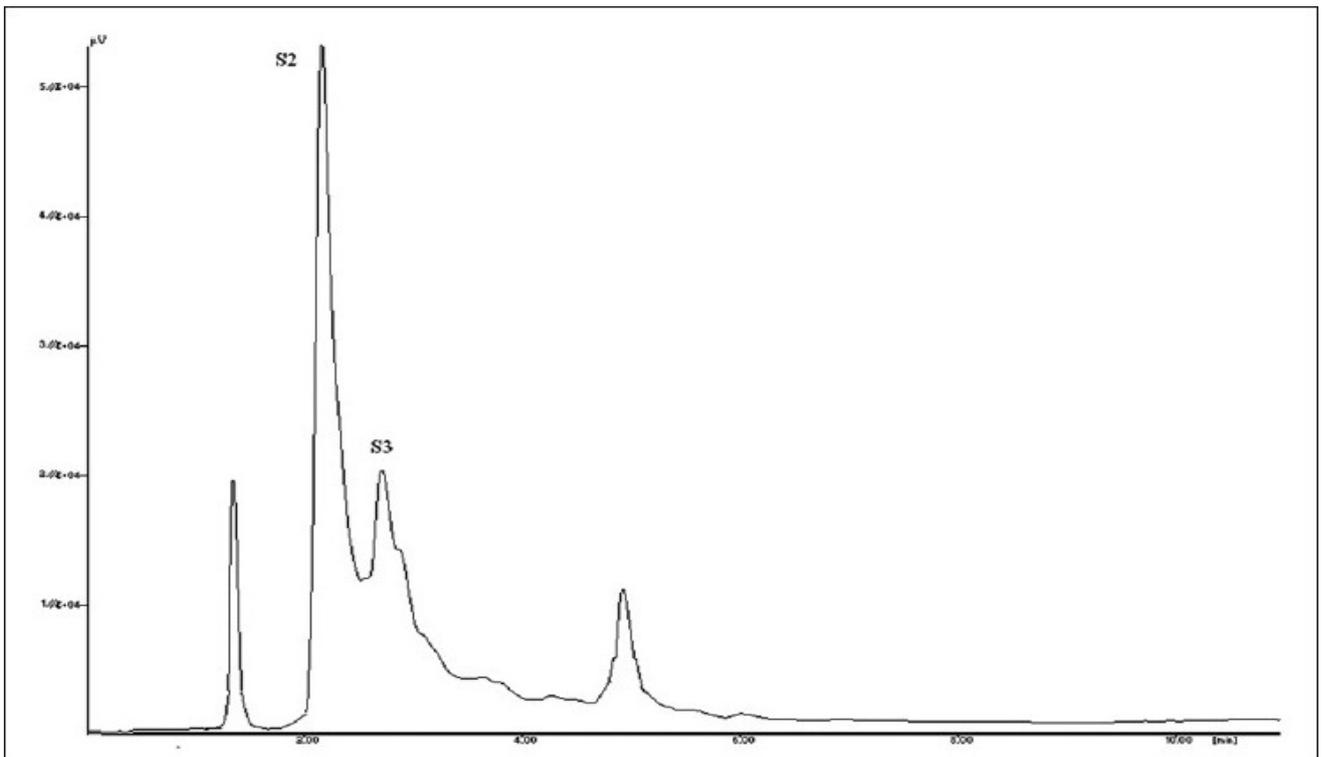


Fig. b: HPLC chromatogram of isolated saponin

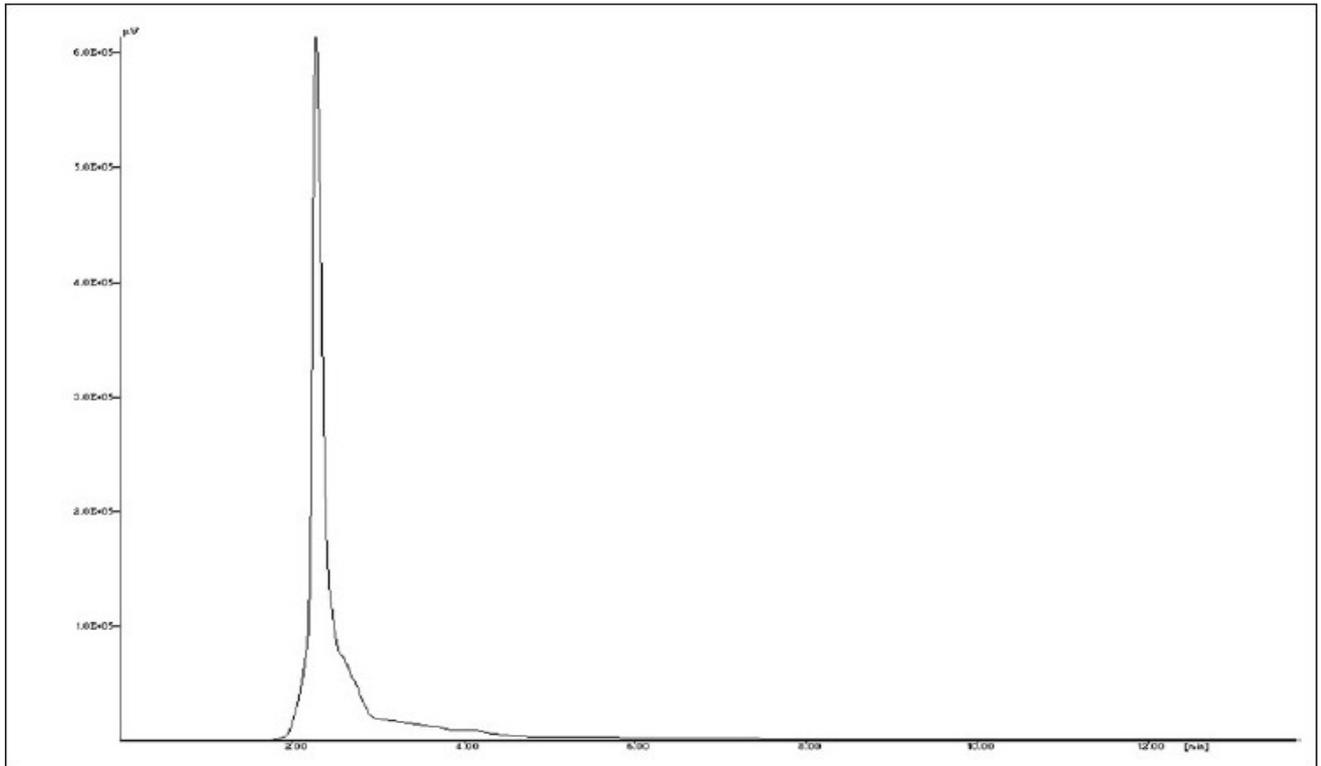


Fig. c: HPLC chromatogram of saponin content of methanolic extract

#### Cell lines and cell cultures

HEp-2, (human epithelial larynx cancer cell line) was obtained from the National Centre for Cell Sciences, Pune, India, and were grown in SMEM media supplemented with 10% FBS 100 IU/ml, penicillin 100 mg/ml, streptomycin 20 mg/ml, kanamycin acid sulphate 20 mg/ml, amphotericin-B, 3% L-glutamine and 7.5% sodium bi-carbonate solution. The cells were maintained as monolayers in 25 cm<sup>2</sup> plastic tissue culture flasks at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> in air. Exponentially growing cells were used in all the experiments.

#### Cytotoxicity and anti-cancer assay

The cell type was seeded at 1X10<sup>5</sup> cells per well in 24-well plates. After overnight growth, the medium was replaced with maintenance medium (SMEM without FBS) containing various concentrations the SFST and incubated for 24 h. The plates were microscopically examined for cytotoxicity. MTT assay was used to assess the cell viability based on its reduction by mitochondrial dehydrogenase enzyme of the viable cells to purple formazan product (Mosmann, 1983). Briefly, cells were diluted in the growth medium and seeded in 24-well plates at 5X10<sup>4</sup> cells/well. After overnight incubation, growth medium was replaced with exposure medium (SMEM without FBS) containing the desired concentrations of SFST. After 24 h, the cells were washed with 200 ml of PBS, and incubated with 100 ml of 500 mg/ml MTT in PBS at 37°C for 3 h. The formazan product was dissolved in 200 ml of DMSO and estimated by measuring the absorbance at 570 nm in an ELISA plate reader. Cell survival was expressed as a percentage of viable cells of treated samples to control samples. The test was performed in triplicate and each experiment was repeated three times. The same protocol was repeated to check the anti-cancer activity of SFST using the known tumorigenic Hep-2 cell lines.

#### Nuclear morphology assay

DAPI Staining was performed as described by Sandra et al (2005) [21] to determine apoptotic cell death. Briefly the cells (5x10<sup>5</sup>) were

seeded into sterilized cover slips in 60mm Petridish for 24hrs & treated with the extract for another 24hrs. Untreated cells & cells treated with DMSO (1%v/v) and H<sub>2</sub>O<sub>2</sub> (0.0014%v/v) were used as controls. Treated cells & controls were rinsed with phosphate buffered saline (PBS) fixed with ice cold 10% Trichloroacetic acid and further washed with cold 70%, 80% ,90% and absolute ethanol. The cells were permealized with Triton X (10% U/V) and stained with 1µg/ml 4,6-diamidino-2-phenylindole (DAPI) for 4min to reduce the background, the stained cells were washed with PBS and were cover slipped with 90% glycerol and observed under a fluorescence microscope (Zeiss Axio Observe, Gott Ingen Germany). Nuclear morphology of apoptotic cells with condensed/fragmented nuclei was examined under a fluorescent microscope and at least 1X10<sup>3</sup> cells were counted to assess apoptotic cell death.

#### Cell cycle analysis

Cell cycle distribution and measurement of the percentage of apoptotic cells were performed by flow cytometry [22]. After treatment, floating cells in the medium were combined with attached cells collected by trypsinization. Cells were washed with cold PBS and fixed in 80% ethanol in PBS at 20°C. After 12 h, fixed cells were pelleted and stained with DAPI (50 mg/ml) in the presence of RNase A (20 mg/ ml) for 30 min at 37°C. About 104 cells were analysed in a Becton Dickinson FACS can flow cytometer. Cell cycle histograms were analysed using Cell Quest software. Apoptotic cells were distinguished by their decreased DNA content, as shown by their weaker staining intensity in the area of the sub-G<sub>0</sub>/G<sub>1</sub> phase.

#### DNA Fragmentation

Cells were lysed with lysis buffer (10 mM Tris-HCl, 5 mM EDTA, 200 mM NaCl, 0.2% SDS and incubated at 60°C for 5 min the sample was digested with 2.5 µl of proteinase K (more than 3 µl<sup>-1</sup> - Sigma) and 5 µl of RNase A(1u µl<sup>-1</sup>) (Fermentas) and was further incubated at 60°C for 1 hour. After this 250 µl of 5M NaCl was added and mixed and then incubated on ice for 5 min to precipitate proteins cells were

than centrifuged for 15min at 10,000 rpm and the supernatant was transformed to a fresh tube, to which an equal volume of isopropanol was added to precipitate the DNA the sample was centrifuged for 10min at 10,000rpm. The supernatant was then discarded and the pellets were washed with cold ethanol. DNA sample was electrophoresed on a 15% agarose gel for 1 hour and 30 min at 10v finally the gel was examined under UV light following ethidium bromide staining to determine apoptotic DNA fragmentation.

#### Nitric oxide (Griess nitrite) assay

Nitric oxide produced during SFST treatment was estimated spectrophotometrically as a formed nitrite ( $\text{NO}_2$ ) by the method of Green et al. (1982) [23] measure the nitrite content, 100 $\mu\text{l}$  of the culture supernatant was incubated with 100 $\mu\text{l}$  of Griess reagent (1% sulphanilamide in 0.1mol/l HCl and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride at room temperature for 10 min. Then, the absorbance was measured at 540 nm using a microplate reader. The nitrite content was calculated based on a standard curve constructed with  $\text{NaNO}_2$  and the nitrite content is expressed as nmoles/ $10^6$  cells.

#### Determination of reduced glutathione

Reduced glutathione (GSH) was determined by the method of Moron (1979) [24]. From a cell suspension with known cell density 0.5 ml was precipitated with 5% trichloroacetic acid. The contents were mixed well for complete precipitation of protein and centrifuged. To an aliquot of clear supernatant were added 2.0 ml of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and 0.2 M phosphate buffer to a final volume of 4.0 ml. The absorbance was read at 412 nm. A series of standards treated in a similar manner were also run to determine glutathione content. The amount of GSH was expressed as nmoles of GSH/ $10^6$  cells.

#### Estimation of superoxide radical generation

The level of superoxide radical generated was estimated by the method described by Babior et al. (1973) [25]. The superoxide levels are expressed as nmoles of ( $\text{O}_2$ ) liberated/ $10^6$  cells.

#### Statistical analysis

Statistical analysis of the data was performed with Student's t-test. Differences with P-values were considered to be statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.0001$  versus control).

### RESULTS

#### SFST induces Cytotoxicity in Laryngeal cancer cells

The cytotoxicity assay (MTT assay) for SFST against HEP 2 cell lines at different concentrations determined the IC<sub>50</sub> (50% growth inhibition) values (Figure 1). An augment in the % growth inhibition with increasing concentration of MEST (400, 800 and 1000  $\mu\text{g/ml}$ ) on HEP2 cell lines was determined. Among these concentrations 1000  $\mu\text{g/ml}$  of SFST showed the maximum growth inhibition on HEP2 cell lines. Although not significant, these concentrations of

SFST had minor effects on control cell line (Figure 1). For all further experiments, IC<sub>50</sub> values were used.

#### SFST induces morphological changes in Laryngeal cancer cells

The cytopathic effect of SFST on HEP-2 cells were analysed using an optical microscope and are presented in Figure 2a, & 2b. Following treatments with SFST extracts (400, 800, and 1000  $\mu\text{g/ml}$ ) for 24 hr, the cells became rounded, hydropic, vacuolated and lost cell contacts when compared to the control cells. In particular, surface morphological changes leading to cell detachment were observed with increasing concentrations of SFST (1000  $\mu\text{g/ml}$ ) (Figure 2b). Control cells appeared elongated, attached smoothly on the culture surface (Figure 2a).

#### SFST induces apoptosis in Laryngeal cancer cells

In the present investigation after 24 hr exposure to SFST, the cells exhibited characteristic apoptotic features in a dose dependent manner (400, 800, and 1000  $\mu\text{g/ml}$ ) such as chromatin condensation and intensely fluorescent nuclear condensation as observed under fluorescence microscope using DAPI staining (Figure 3b & 3c). In contrast, the control cells were intact and did not exhibit fluorescence (Figure 3a).

#### SFST induces cell cycle changes in laryngeal cancer cells

Flow cytometric analysis of exponentially grown cells treated with IC<sub>50</sub> of SFST was performed to investigate whether the SFST affected cell cycle regulation. As shown in (Figure 4a, 4b & 4c), HEP-2 cells significantly reduced the DNA content, making them appear in the sub-G<sub>0</sub>/G<sub>1</sub> or A<sub>0</sub> region indicative of apoptosis, with consequent loss of cells in the G<sub>1</sub> phase upon treatment with SFST. SFST increased the number of sub-G<sub>1</sub> population (hypodiploid cells) 12 h after treatment (14.8%), which referred to the cells that underwent apoptosis. After 24 h, the number of sub-G<sub>1</sub> cells was markedly increased and reached the peak level at 98.8%.

#### SFST induces DNA fragmentation in Laryngeal cancer cells

DNA fragmentation assay was performed to confirm the results of the MTT assays and flow cytometric analysis. SFST treatment resulted in the degradation of chromosomal DNA into small inter nucleosomal fragments, as evidenced by the formation of 180-200 bp DNA ladders on 2% agarose gels which is characteristic of apoptosis. HEP-2 cells exhibited a clear and marked DNA ladder pattern at 24 h after SFST exposure and increased over time as shown in (Figure 5).

#### SFST generates changes in free radicals and GSH levels in Laryngeal cancer cells

As shown in Figure. 6, incubation of HEP-2 cells with SFST for 24 hr resulted in dose-dependent decrease in nitrite generation by the cells, whereas the levels of superoxide production were significantly increased in SFST -treated cells when compared to control cells (Figure. 7). The level of glutathione also significantly decreased when compared to untreated cells (Figure. 9).

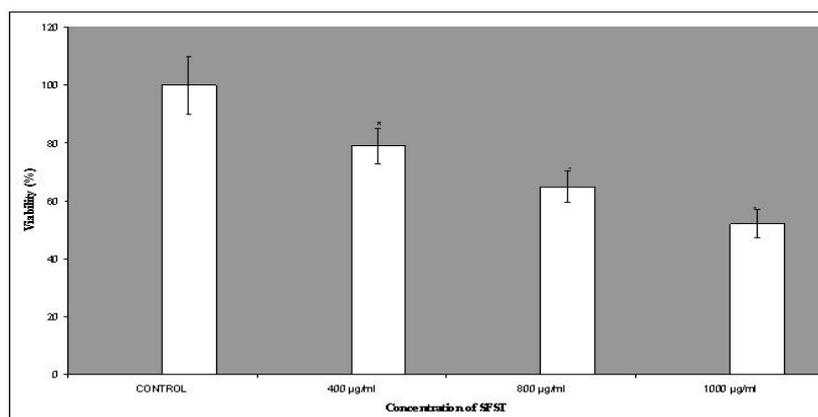
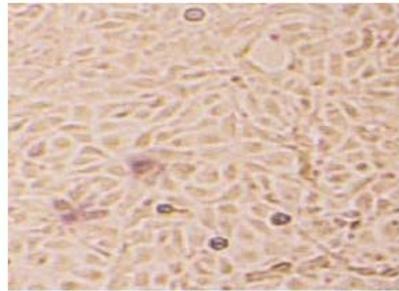
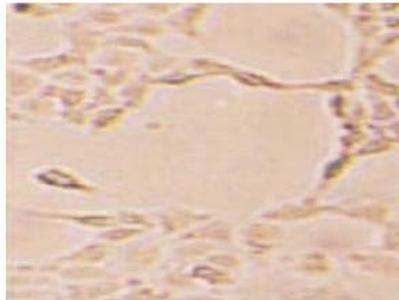


Fig. 1: Cytotoxicity in HEP-2 cells induced by exposure to different concentration of SFST as determined by MTT reduction assay

The columns represent the percentage of viability in HEp-2 cells. Cell viability is shown as mean  $\pm$  S.D. derived from at least three separate experiments done in triplicate. The values are represented as mean  $\pm$  S.D. \*P < 0.0001 versus control

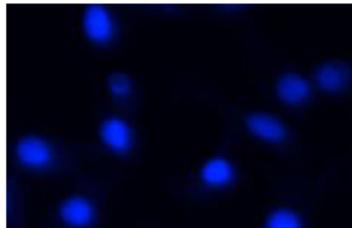


**HEp-2 control cells**

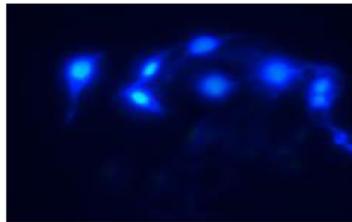


**HEp-2 SFST treated cells (1000 µg/ml),**

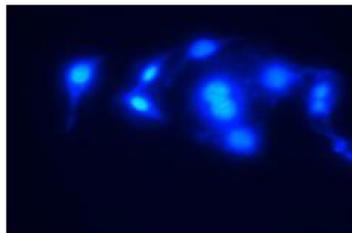
**Fig. 2: Light microscopy photographs of untreated HEp-2 control and SFST treated cells (1000 µg/ml), for 24 h**



**(3a) Control HEp-2 cells**



**(3b) HEp-2 cells treated with SFST (400 µg/ml)**



**(3c) HEp-2 cells treated with SFST (1000 µg/ml)**

**Fig. 3: Effect of SFST extract on nuclear morphology (DAPI staining) of HEp-2 Cells STST induced morphological changes characteristic of apoptosis (chromatin condensation and nuclear shrinkage) seen at all concentrations**

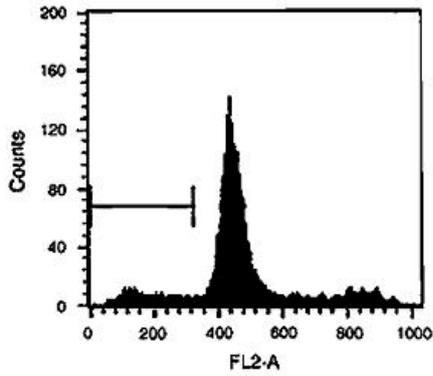


Figure 4a control Hep-2 cells

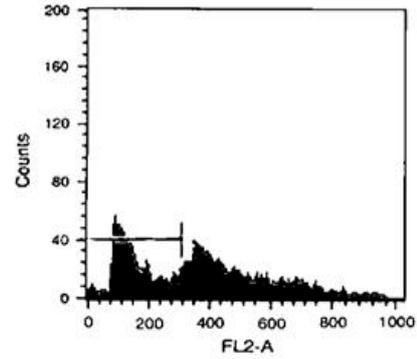


Figure 4b Hep-2 cells treated with SFST (1000 µg/ml)

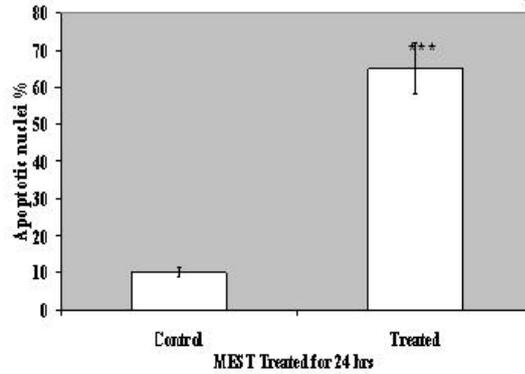


Figure 4c

Fig. 4: Cell cycle analysis of control Hep-2 cells Figure 4a and Figure 4b Hep-2 Cells treated with SFST

Representative histograms demonstrate the cell population according to the DNA content determined by DAPI staining. Figure 4c represents the Quantitative analysis of apoptotic Cell population in Htp-2 Cells Error bar represents SD between counts of three independent experiments. \*\*\*e significantly increased compared to control ( $p < 0.001$ ) by ANOVA followed by Tukey-HSD test.

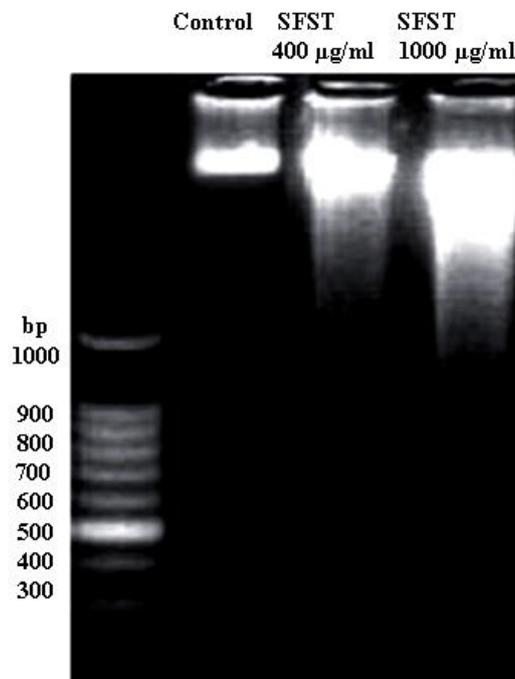
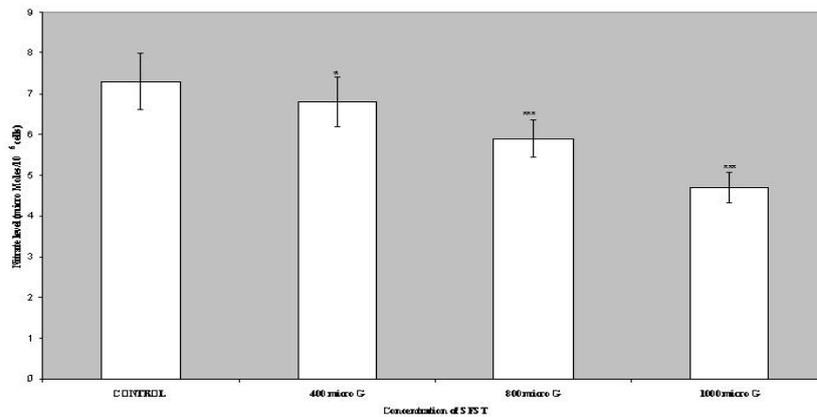
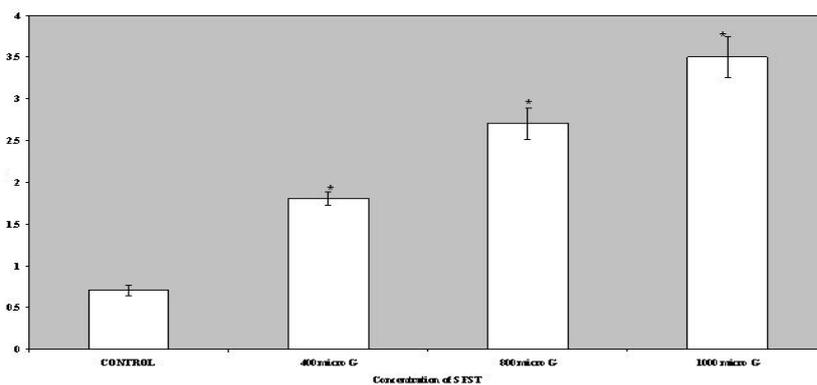


Fig. 5: Analysis of DNA fragmentation by Agarose gel electrophoresis. Lane I: DNA marker, Lane 2: Control, Lane 3: HEp-2 Cells treated with SFST 400 µg/ml and Lane 4: HEp-2 Cells treated with SFST 1000 µg/ml



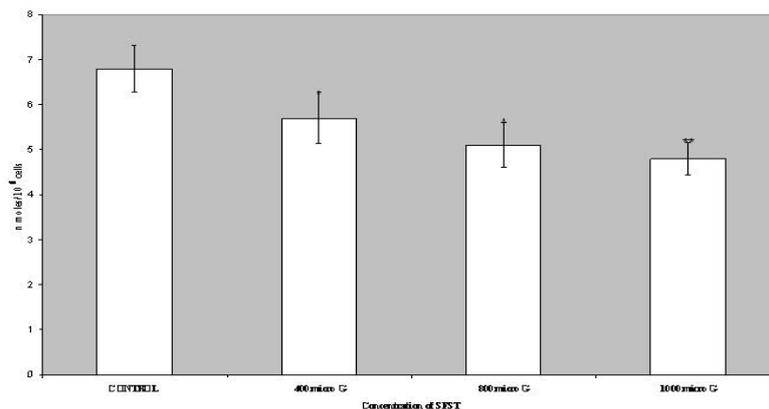
**Fig. 6: Effect of different doses of SFST on the levels of nitrate in the HEP-2 Cells.**

The columns represent the  $\mu\text{M}$  of nitrate level in 10<sup>6</sup> in HEP-2 cells. The values are represented as mean  $\pm$  S.D. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.0001 versus control



**Fig. 7: Effect of 24 hr treatment with different doses of SFST on the levels of superoxide production in the HEP-2 cells**

The columns represent the n moles of superoxide production in HEP-2 cells. The values are represented as mean  $\pm$  S.D. \*P < 0.0001 versus control



**Fig. 8: Effect of 24 hr treatment with different doses of SFST on the levels of glutathione in the HEP-2 cells**

The columns represent the n moles of glutathione in HEP-2 cells. The values are represented as mean  $\pm$  S.D. \*P < 0.01, \*\*P < 0.0001 versus control

## DISCUSSION

A successful anticancer compound should kill or incapacitate cancer cells without causing excessive damage to normal cells. Certain products from plants are known to induce apoptosis in cancer cells but not in normal cells. Thus, it is important to screen apoptotic inducers from plants, either in the form of crude extracts or as active isolated components. A growing body of evidence has emerged, based on many studies, suggesting that products derived from plants

are useful in the treatment as well as in the prevention of cancer. However plant phytochemicals serve to continue as a viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [26].

Thus phytochemicals are extensively used as chemopreventive agents capable of inhibiting cell proliferation, inducing apoptosis or signal transduction in *invitro* and animal models [27, 28]. Hence this scenario is under taken to cram the *Solanum trilobatum* steroids

(saponins) as a chemopreventive or chemotherapeutic drug on human laryngeal cells.

The cytotoxicity assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. Thus the results of our study demonstrate that SFST extract is preferentially cytotoxic to human larynx carcinoma cells in a dose dependent manner. Furthermore superoxide generation, reduction in nitrite levels and depletion in intracellular glutathione status also confirmed the cytotoxic effects of SFST.

The morphological and biochemical features of apoptosis were distinct from that of necrosis [29]. Thus incomplete cellular membranes, cellular swelling and vacuole degeneration were clearly evident on examination under fluorescence microscope. These processes often represent Pyknosis of chromatin due to the selective activation of an endogenous endonuclease when viewed under fluorescence microscope [30]. Surh, 1999 has reported that apoptosis was found to be the mode by which several natural phenolic compounds induce cell death in malignant cancer cell lines. The presence of different classes of phenolic compounds (steroidal glycoalkaloids such as sobatum,  $\beta$ -sitosterol, disogenin and  $\beta$ -solamarine, polyphenols like quercetin, and tannins, and steroids like saponins in the methanolic extract of *Solanum trilobatum* [31] is thus accountable for the induction of apoptosis in the laryngeal cancer cells in our study.

To probe the possible mechanism by which SFST enhances the cytotoxic effects, we performed some experiments on cell cycle distribution and confirmed the extent of apoptosis. As an outcome of flow cytometry analysis the administration of SFST (250mg/ml) indulges the accumulation of cells at G2/M phase validating the extent of apoptosis. Gao et al. 2006 [32] described in his study that flavanoids present in *Actaea asiatica* was effective against MCF-7 cell line, and also to hepatoma cells, inducing apoptosis by G0/G1 cell cycle arrest. Prasanna et al., 2009 [33] also evidently confirmed that the phytochemicals screened in *Cassia auriculata* leaf extract inhibited the proliferation of MCF-7 and Hep-2 cells through stimulation of apoptosis. On the contrary, in our research, as well as in studies by (Kauslmann and Earnshaw 2000) [34] such phenomena were not found in normal cancer cells. Our results revealed that SFST -induced apoptosis was not triggered at a specific phase of the cell cycle. SFST - did not affect the cell cycle profile; however, it increased the number of apoptotic cells (sub-G1 populations) in a time-dependent manner. Consistent with the MTT assay and flow cytometric analysis Hep-2 cells treated with SFST -demonstrated the highest sub-G1 population and cleared the appearance of DNA fragmentations at 24 h post treatment, which is known as a hallmark of apoptotic cell death. Both DNA loss and a decrease of DNA accessibility to the dye are responsible for the lower fluorescence of apoptotic cells [33], making them appearing in the sub-G0/G1 region of the histogram. The appearance of such fragments resulting in a ladder formation was evident when fragmented DNA free of genomic DNA was isolated from cells treated with SFST and subjected to agarose gel electrophoresis.

Extracts of various medicinal plants as well as plant phenolics, particularly polyphenolic flavonoids and certain alkaloids have been investigated for a positive apoptotic response and induce cell death in a variety of human malignant cancer cell lines [35]. Epigallocatechin-3-gallate, a flavonoid rapidly inhibited proliferation of HL-60 cells, which interrelated with the formation of apoptotic DNA fragments [35]. Kaur et al. (1992) [36] illustrated the anti-proliferative and cytostatic effect of a polyhydroxylated flavonoid in vitro, which displayed tough inhibition of DNA, RNA and protein synthesis and selectively obstructed cell cycle progression in vitro. Saponins and tannins [37, 31] the bioactive plant phytochemicals present in SFST possessed the antitumor effects by cell growth inhibition and apoptosis in a variety of cancer cells [38].

Apoptosis is activated either by the generation of oxygen free radicals or by the decline in the levels of endogenous antioxidants [39]. The signaling cascade leading to programmed cell death seems to involve ROS as second messengers [40]. Today, a large body of evidence suggests that oxidative stress induces apoptosis and that a concomitant increase of oxygen free radicals might function as a cellular messenger [41]. Moreover free radicals are also involved in tumor promotion through growth of cancer cells by enhancing the expression of somatic mutation and by interference in the extra cellular processes that normally inhibit cancer cell growth [42]. Our study is in accordance with the work demonstrated by Vijaya Padma et al. (2007) [43] that revealed the involvement of reactive oxygen species to induce apoptosis in Hep-2 cells. As such a dose dependent increase in the superoxide and decrease in nitric oxide levels were observed significantly in the Hep2 cells treated with SFST when compared with control cells. On the other hand we investigated the levels of cellular GSH a tripeptide and responsible for hydrophilic xenobiotics conjugation. Sulphydryl group of glutathione is essential for its antioxidant activity against some forms of reactive oxygen species (ROS) in cells. Reduction of antioxidant levels has been shown to enhance susceptibility to oxidative stress-induced cytotoxicity [43]. As such our study confirmed that SFST exerted a dose dependent inhibition in the intracellular GSH levels in Hep-2 cells when compared to the untreated cells.

## CONCLUSION

Therefore in conclusion our study established that SFST inhibits the proliferation of Hep-2 cells through induction of apoptosis and by enormous production of free radicals and reduced amount of GSH expression. Even though additional studies are needed to validate *Solanum trilobatum* saponin as a candidate of anti-cancer drug, the current work opens a new standpoint for cancer therapy based on ethnopharmacological studies.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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