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# A Review of *In Vitro* and *In Vivo* Anti-oxidant and Anti-Cancer Activities *of Gymnema Sylvestre*

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## ABSTRACT

A plant of Apocynaceae family, *Gymnema sylvestre,* is used as a traditional therapy for various purposes and also, as a dietary supplement because it has numerous therapeutic benefits. In this paper, the studies on *Gymnema sylvestre* and its bioactive components effect on oxidative stress and human cancer cell *in vitro* and *in vivo* models are reviewed. Due to the presence of bioactive compounds in *Gymnema sylvestre* such as flavonol glycoside, lupeol, sterols, triterpenoid saponins, dammarene saponins and triterpene saponins it can act as a promising cytotoxic and anticancer agent against several human cancer cell lines. Therefore, *Gymnema sylvestre* have been shown to be effective against cancer development and progression and oxidative stress, and should be considered safe and effective to use in cancer prevention and therapy and as an antioxidant.

**Keywords:** *Gymnema sylvestre*, Anti-cancer, Anti-oxidant, Free radicals cytotoxic

## INTRODUCTION

Various natural plant and herbal medicines, functional foods, and phytomedicines have been studied by scientists to develop effective and beneficial therapeutic potentials. Some of these include antidiabetic [1-4], anticancer [5,6], immunomodulating [7-11], antiobesity, and improving lipid profile [1,12], anti-inflammatory [13] and anti-bacterial [14] effects. One of the natural plants products with medicinal potentials that can be used for treating various diseases is *Gymnema sylvestre*, which is a member of the Apocynaceae family. This plant can be found in various countries, such as India, Australia, and China. It is also known as 'Gurmur', because of its Blood glucose lowering properties. In an attempt to understand the molecular mechanism of genes responsible for medicinal properties of Gymnema sylvestre, two partial cds (accession nos. GU191124; GU181368) were submitted to NCBI database by Tiwari *et al.* [15]. They have concluded that further studies into the identification and characterization of genes involved in the biosynthesis of triterpene glycosides, gymnemic acids would provide valuable information in deciphering the biosynthetic pathway of gymnemic acids and the mechanism of their pharmacological activities in the plant. The putatively pathway for triterpene glycosides is derived from the isoprenoid pathway with glycosylation of the triterpene aglycone at the terminal transformation of gymnemagenin. A general diagrammatic sketch has been drawn to represent a putative pathway with a focus on terminal pathway steps in biosynthesis of saponins from Gymnema sylvestre. Further, in Fig. 7 it was assumed that gymnemagenin (sapogenin) gave rise to gymnemic acids and derivatives through glycosylation mechanism by glycosyltransferases [15]. This natural plant is considered as one of the main botanicals to treat diabetes in the natural system of medicine, originated in India and also included in Indian Pharmacopoeia as an anti-diabetic plant [16].

*Gymnema sylvestre* can also be used against other most important diseases, such as cancer and cardiovascular diseases, asthma, diabetes [17] and obesity [18,19]. Oxidative stress, which is defined as an excess of reactive oxygen species (ROS) in comparison to antioxidants, has been associated with neurodegenerative illness, cardiovascular disease, diabetes mellitus, and a variety of other diseases [20]. These findings highlight the need to strike a balance between the relative abundance of ROS and

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antioxidants. To maintain this equilibrium, cells contain complicated biochemical and genetic pathways, and their disruption can have serious pathological repercussions. Cancer cells have an abnormal redox equilibrium, yet while reactive oxygen species (ROS) are pro-tumorigenic, large quantities of ROS are lethal [21]. Tumor cells hyperproliferation is associated with a large generation of ROS, yet they have evolved to flourish in settings where this oxidative load pushes the redox balance away from a reduced state. To do this, tumor cells increase their antioxidant state to improve ROS-driven proliferation while avoiding ROS thresholds that would cause senescence, apoptosis, or ferroptosis [22,23].

In this review, we investigated a number of pharmacological characteristics of *Gymnema sylvestre*, including anti-cancer and antioxidant effects. Because the anti-proliferative and apoptosis-inducing effects of *Gymnema sylvestre* extracts on various cancer cells had not previously been investigated, this study was undertaken to determine whether *Gymnema sylvestre* extracts would have anti-proliferative consequences as well as mechanisms of cell death elicited by the extract with reducing oxidative stress caused by tumor cells.

## ANTI-CANCER ACTIVITY OF GYMNEMA SYLVESTRE-IN VITRO

Khanna and Kannabiran [24] tested the anticancer activity of isolated saponins, gymnemagenol (C30H50O4) from *Gymnema Sylvestre* and dasyscyphin C (C28H40O8) from Eclipta prostrata leaves *in vitro* in HeLa cells. Gymnemagenol and dayscyphine C at 50 µg $ml^{-1}$ showed good cytotoxic activity (63% and 52%, respectively) in HeLa cells after 48 h with IC50 values of 37 and 50 µg $ml^{-1}$, respectively. 5-Fluorouracil (5-FU), a positive control, showed 57.5 µl of mortality with an IC50 value of 36 µg $ml^{-1}$. HeLa cell death was maximal (73%) after 96 h with gymnemagenol, while dasyscyphin C was only 53%. One of the results acquired was the non-toxic effect of the isolated saponins on Vero cells. Thus, it can be determined that saponins gymnemagenol and dayscyphine C have significant antitumor-cytotoxic activity on HeLa cells under *in vitro* conditions [24].

In the study done by Khanna and Kannabiran [25], the isolated saponins, gymnemagenol (C30H50O4) from *Gymnema Sylvestre* and dasyscyphin C (C28H40O8) from Eclipta prostrata leaves showed non-proliferative activity on HepG2 cells under in vitro conditions. Both gymnemagenol and dasyscyphin C showed non-proliferative activity on HepG2 cells at 24 h with the IC50 value of 18.5 and 23.5 µg $ml^{-1}$ respectively, while 5-Fluorouracil (5FU) which is a positive control showed the IC50 value of 1.34 µg $ml^{-1}$. The isolated active principle gymnemagenol exhibited a high degree of inhibition over the growth of the HepG2 cells when compared to dasyscyphin C. The non-proliferative effect of gymnemagenol and dasyscyphin C was also tested on Vero cells. It was found that both the saponins were less toxic to Vero cells. This study determined that the saponins, gymnemagenol and dasyscyphin C have significant non-proliferative activity in vitro conditions on HeLa cells [25].

Because of the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules, administrating *Gymnema sylvestre* extract to rats increased superoxide dismutase activity and decreased lipid peroxide through directly scavenging the ROS. The gold nanoparticles (AuNPs) were bio-functionalized using the bioactive compounds from the aqueous extract of *Gymnema sylvestre* and characterized by ultraviolet-visible spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray analysis, Fourier transform infrared spectroscopy, and X-ray diffraction by size and shape. The characterized bio-functionalized *Gymnema sylvestre (GGNPS)* were tested for its in-vitro anticancer activity against human colon adenocarcinoma cells. Bio-functionalized *Gymnema sylvestre (GGNPS)* showed the Surface Plasmon Resonance (SPR) band at 540 nm. The SEM Images showed the spherical-shaped nanoparticles at an average of 72.8 nm and further determined using the Scherrer equation. TIt also found that in vitro cytotoxic activity of the bio-functionalized GGNPs indicates the sensitivity of the human cancer cell line for cytotoxic drugs which is higher than that of Vero cell line for the same cytotoxic agents. Furthermore, the results suggest that the medicinal properties of the bioactive compounds by bio-functionalize can be increased with AuNPs without compromising their medicinal properties and this can also be a good alternative method to obtain AuNPs with improved anticancer properties [26].

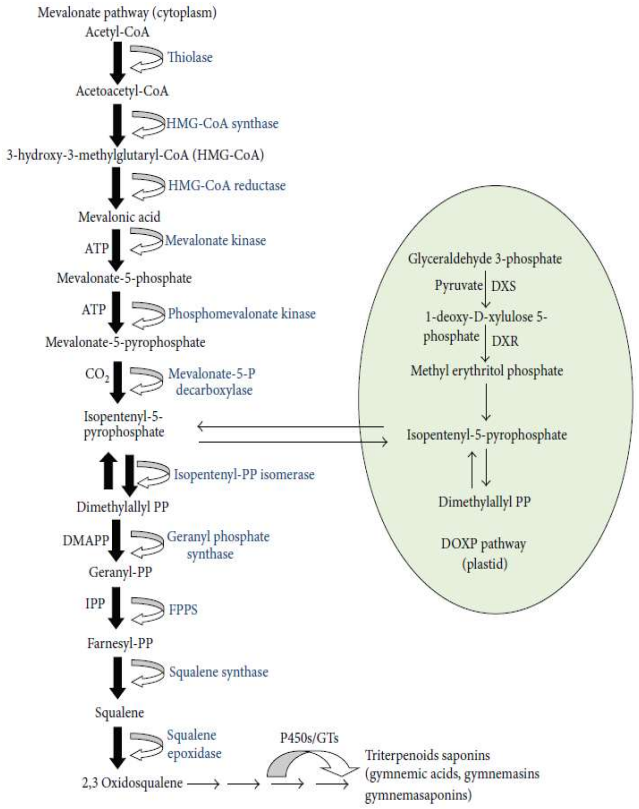


**Fig. 1.** Hypothetical pathway of Gymnemic acid biosynthesis, representing the general sketch for the formation of triterpenoids through Mevalonate pathway [15].

The biological synthesis of silver (GYAgNPs) and gold (GYAuNPs) nanoparticles from *Gymnema sylvestre* leaf extract and their *in vitro* free radical scavenging efficacy as well as anti-proliferative effect in Hep2 cells was reported by Nakkala *et al.* [27]. The formation of silver and gold nanoparticles was confirmed by UV-Vis spectroscopy. The average size of synthesized GYAgNPs and GYAuNPs was found to be 33 and 26 nm, respectively, by DLS particle size analyzer. TEM analysis indicated the spherical shape of GYAgNPs and GYAuNPs and in EDX analysis they produced strong signals for silver and gold, respectively. Both GYAgNPs and GYAuNPs exhibited strong in vitro free radical quenching ability and their activity was comparable to that of GYLE. The cytotoxic effect of GYAgNPs and GYAuNPs in Hep2 cells was examined by MTT assay in which GYAgNPs displayed an IC50 value of 121 l g $ml^{-1}$, while GYAuNPs produced up to 38% of inhibition at the maximum concentration of 250 l g $ml^{-1}$ used in this study. Distinct morphological changes were observed in Hep2 cells following treatment with GYAgNPs and GYAuNPs at 24 h, and orange-colored apoptotic bodies were located by acridine orange and ethidium bromide double-staining technique. Also, there was an increase in the levels of reactive oxygen species in treated cells as indicated by 20, 70-dichlorofluorescin diacetate staining. Further, nuclear changes like chromatin condensation/fragmentation were also observed by propidium iodide and 40, 6-diamidino-2-phenylindole dilactate staining methods. These findings support that the anti-proliferative effects of GYAgNPs and GYAuNPs in Hep2 cells are mediated through induction of apoptosis [27].

Arunachalam *et al.* [28] tested the preliminary phytochemical screening for bioactive compounds from aqueous extracts and revealed the presence of alkaloids, triterpenes, flavonoids, steroids, and saponins. The characterized bio-functionalized *Gymnema* Sylvestre were tested for its in vitro anticancer activity against HT29, a cell line with epithelial morphology, human colon adenocarcinoma cells. In vitro cytotoxic activity of the bio-functionalized green-synthesized SNPs (GSNPs) indicated that the sensitivity of HT29 human colon adenocarcinoma cells for cytotoxic drugs is higher than the bioactive compound of the aqueous extract. The results of this study specifically showed that the anticancer properties of the bioactive compounds *Gymnema* Sylvestre can be enhanced through biofunctionalizing the SNPs using the bioactive compounds present in the plant extract without compromising their medicinal properties [28]. Figure 2 confirmed thin vitro cytotoxicity effect of GYAgNPs (silver nanoparticles from Gymnema sylvestre leaf extract) and GYAuNPs (gold nanoparticles from *Gymnema sylvestre* leaf extract) against Hep2 cells and HaCaT cells based on the MTT test results [28].

## ANTIOXIDENT ACTIVITY OF GYMNEMA SYLVESTRE-IN VITRO

A study was designed to examine chitosan and a mixture of chitosan, ascorbic acid and *Gymnema Sylvestre* for its antioxidant and hypoglycemic activities in hypercholesterolemic rats. Plasma lipid concentrations,

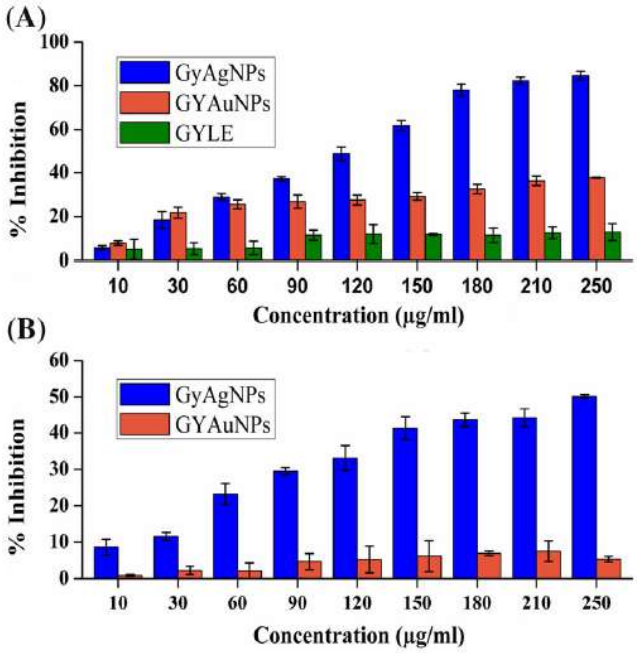


**Fig. 2.** *In vitro* cytotoxicity effect of GYAgNPs (silver nanoparticles from Gymnema sylvestre leaf extract) and GYAuNPs (gold nanoparticles from Gymnema sylvestre leaf extract) against (a) Hep2 cells and (b) HaCaT cells [28].

transaminases (ALT and AST), lactate dehydrogenase (LDH) activity, glucose, malondialdehyde (MDA) and total blood reduced glutathione (GSH), superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) in erythrocytes and plasma glutathione reductase (GR), glutathione-S-transferase (GST) and catalase (CAT) were examined. Dietary causes of hypercholesterolemia are demonstrated by increases in total lipids (TL), total cholesterol (TC), triglycerides (TG), LDL-C levels, ALT, AST, LDH and MDA levels, as well as GSH and enzyme depletion. By improving antioxidant enzymes activities, antioxidant GSH content was significantly increased while lipid peroxide level MDA was significantly decreased in the treated groups compared with the hypercholesterolemic (HC) group due to the hypolipidemic and hypocholesterolemic effects of chitosan, ascorbic acid and *Gymnema sylvestre*. These results suggest that the cholesterol-lowering effects of the supplement blend, chitosan, ascorbic acid and *Gymnema Sylvestre*, may be an appropriate mixture to reduce oxidative stress, possessing the ability to lower plasma cholesterol levels as well as slow lipid peroxidation and enhance antioxidant enzyme activity [29].

Elicitation is one of the few strategies that find commercial application in the improvement of secondary metabolite production from plants as well as cell and organ culture systems. *Gymnema sylvestre* is an important medicinal plant which bears bioactive compound namely gymnemic acid that finds application in the treatment of diabetes. The study conducted by Praveen *et al.* [30] relates to different polyunsaturated fatty acids (PUFAs) as elicitors to enhance biomass accumulation and gymnemic acid production in hairy root cultures of *Gymnema sylvestre*. Hairy root cultures of *Gymnema sylvestre* were elicited with oleic and linolenic acid at 0, 1, 5, 10 and 50-M concentrations respectively. Elicitors were added to the hairy root cultures on 15th day of culture and the roots were harvested on day 20. Cultures supplemented with 1 and 5-M linolenic acid enhanced the gymnemic acid content significantly (45.10 ± 0.11 mg g-1DW) and (87.99 ± 0.53 mg g-1DW) compared to that of control (11.30 ± 0.20 mg g-1DW) respectively without the decrease of hairyroot biomass. An increase of 7.78 fold increment of gymnemic acid content was evident with 5-Mlinolenic acid compared to that of the control. Supplementation of oleic acid also increased the production of gymnemic acid content in hairy root cultures but not in considerable amounts compared with the linolenic acid treatments. Changes in the total phenols, flavonoids and antioxidant activity were evident upon elicitation with PUFAs. This was the first report on linolenic acid as an elicitor for gymnemic acid production in *Gymnema sylvestre* hairy root cultures [30].

Herbal curd was prepared by adding the *Gymnema sylvestre* leaf extract during fermentation and its activity against liver cancer was investigated on HepG2 cell lines by MTT (3-[4,5-dimethylthiazole-2yl]-2,5 diphenyltetrazolium-bromide) assay. The prepared herbal curd was characterized with the help of qualitative phytochemical analysis, Fourier transform infrared (FT-IR) spectroscopy and gas chromatography mass spectrometry (GC–MS). The sensory attributes and stability of the herbal curd were also determined. The qualitative phytochemical analysis of the herbal curd showed the transfer of phenolic compounds from the plant extract to herbal curd. The FT-IR analysis exhibited that the Anhydride group present in the plant extract was infused into the herbal curd in addition to its other functional groups such as Alcohol, Alkyne, Alkene, Amide, Sulfate,

Ether and Esters. The GC–MS analysis revealed the presence of 10 new compounds which were not present in curd or plant extract. The results of MTT assay showed that the herbal curd had significantly reduced the growth of HepG2 cells. The sensory evaluation proved that the herbal curd was delicious than the plant extract. Hence, the herbal curd can be used as a potential food supplement for the treatment of liver cancer [31].

Santhoshkumar *et al.* [32] conformed the biological synthesis of ZnO NPs from *Gymnema sylvestre* initially by the color change of the reaction mixture from colorless to brown color, indicating the synthesis of ZnO NPs preliminarily. Then, the synthesized nanoparticles were exhibits as strongest UV absorbance peak at 300 nm. Moreover, the particle size of the synthesized nanoparticles was analyzed by Zeta potential and particle size analysis. The reported synthesis of $Mg^{2+}$ doped ZnO NPs using the leaf extract of *Gymnema sylvestre*. The presented peaks were directly equivalent to protein and enzymes molecules or polysaccharides which are found in the cell biomass. The presented result implies that the products comprised of pure phases. Moreover, the effective diffraction peaks were found more rigorous and narrower that implying a respectable crystalline structure of Zn nanofabricated products. The respectable of size range of ZnO nanofabricated material was from 20 nm to 100 nm. As the experiment by Santhoshkumar et al. indicates the surface and shape with size morphology of ZnO were characterized from the microscopical studies of SEM. This study evident that ZnO NPs were spherical and irregular in shape and were poly-dispersed. The measured average size was 50μm, occasional agglomeration of the ZnO NPs has been observed. These all the characterization studies are scientifically evident that present nanoparticles are ZnO. ZnONP-GS treatment associated morphological changes of apoptotic MCF-7 cells were test by double staining of acridine orange and ethidium bromide staining. It was noticed ZnONP-GS treated cells showed a greater number of apoptotic cells significantly in dose depended manner. Conversely, untreated MCF-7 cells showing there is no significant apoptotic cells conformed by green fluorescence staining. ZnO NPs treatment arbitrated apoptotic protein appearance was studied using western blotting analysis. As the results of this study confirms an extensively increased protein expression of Bcl-2 and broadly decreased expression of Bax, caspase-9 and 3 protein in untreated breast cancer cells. This result indicates that ZnONPs-GS induces pro-apoptotic mediator's leads to cell death [32].

Rotondo *et al.* [33] investigated the effect of *Gymnema sylvestre* extract on human glioblastoma cells U87Mg. Glioblastoma is a brain tumor, characterized by recurrent or innate resistance to conventional chemo radiotherapy. Novel natural molecules and phyto-extracts have been proposed as adjuvants to sensitize the response to Temozolomide (TMZ) According to the IC50-values, *Gymnema sylvestre* extract displayed a significant cytotoxicity. *Gymnema sylvestre* induced reduction in Pro-caspase 9, 3, but not PARP cleavage nor DNA fragmentation. Thus, in GS-induced cytotoxicity, cell death is not associated with apoptosis. In this context, short-term treatment of U87Mg cells with *Gymnema sylvestre* extract (1 mg $ml^{-1}$) reduced the phosphorylation levels of mTOR and of its downstream target P70 S6 kinase, highlighting the role of *Gymnema sylvestre* extract into autophagy induction. The activation of autophagic flux by *Gymnema sylvestre* extract was confirmed by Western blot analysis, which revealed the reduction in p62 and the concomitant increase in LC3B II/I ratio. Immunofluorescence evidenced the accumulation of LC3B puncta in U87Mg cells pretreated with autophagy inhibitor Bafilomycin A1. Furthermore, as main key regulators of type II programmed cell death, p53, p21 and CDK4 were also investigated and were inhibited by *Gymnema sylvestre* treatment. Figure 3 shows the morphological variations of U87Mg human glioblastoma cells after 24, 48, and 72 h of exposure to *Gymnema sylvestre* 0.5 and 1 mg $ml^{-1}$]. In Fig. 4, p53 expression was evaluated by Western blot analysis, which revealed a strong reduction in this protein after 72 h of exposure to *Gymnema sylvestre* extract (Fig. 4A). Alongside this, the expression of autophagy associated protein p62 showed more than 50% of reduction (Fig. 4B). In conclusion, *Gymnema sylvestre* extract could be considered as an autophagy inducer in glioblastoma cells U87Mg [33].

## ANTICANCER AND ANTIOXIDANT ACTIVITY OF GYMNEMA SYLVESTRE-IN VIVO

The ethanol extract of *Gymnema Sylvestre* leaves was examined *in vitro* and *in vivo* to study the role of antioxidants

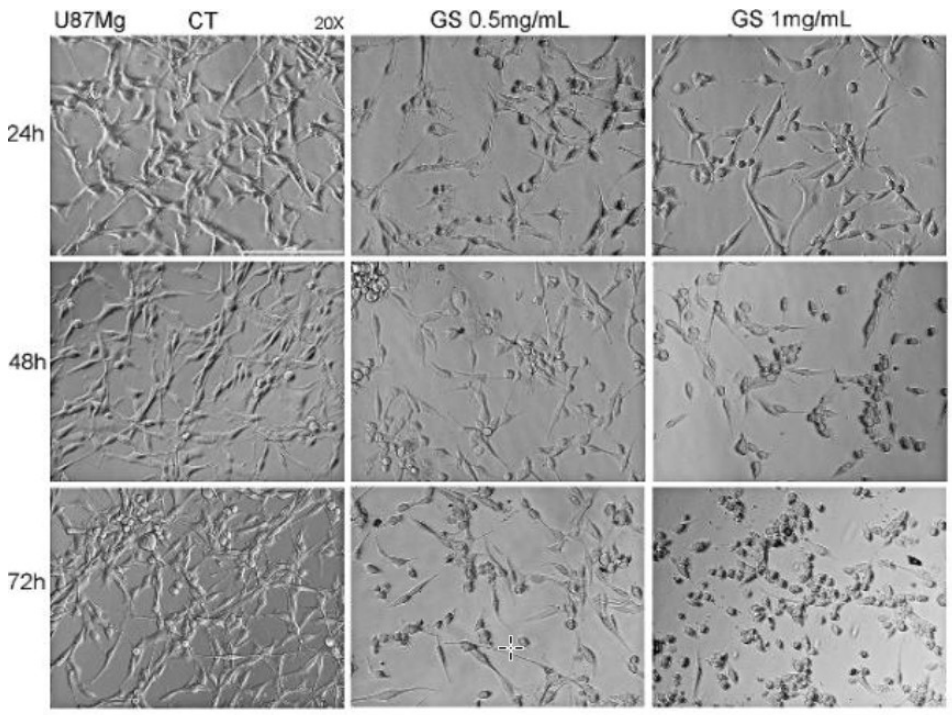


**Fig. 3.** Morphological variations of U87Mg human glioblastoma cells after 24, 48, and 72 h of exposure to *Gymnema sylvestre* 0.5 and 1 mg $ml^{-1}$ [33].

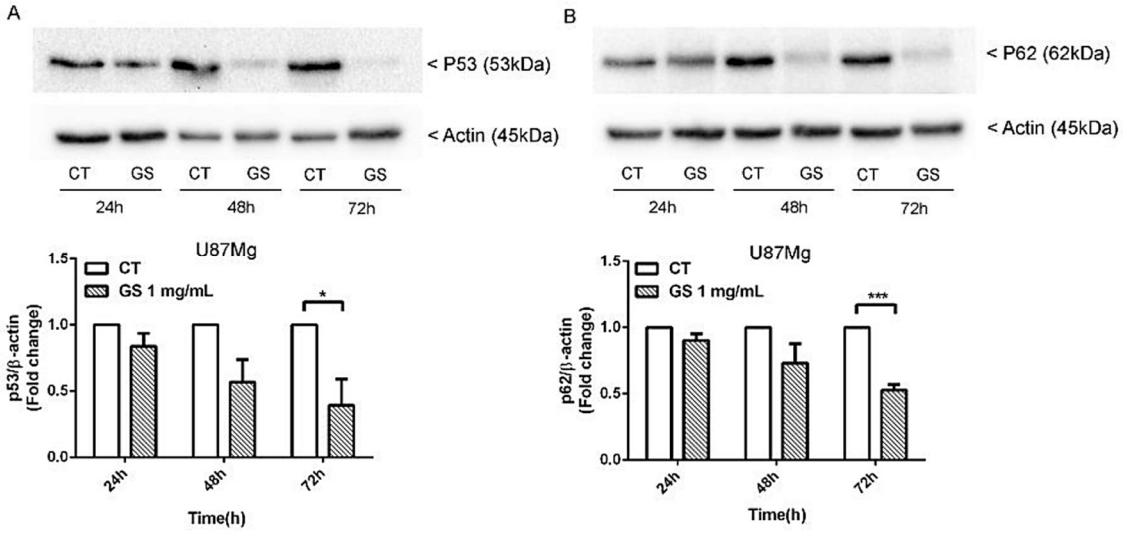


**Fig. 4.** Autophagy modulation by *Gymnema sylvestre* extract [33].

in diabetic rats [34]. The extract showed strong antioxidant activity in tests including TBA (56%), SOD-like (92%) and ABTS (54%). The results obtained from the antioxidant tests in the present study indicate that *Gymnema sylvestre* leaves contain powerful antioxidants. Phenolic compounds found in natural plants are known to have a number of beneficial health effects associated with natural antioxidants, such as preventing the oxidation of LDL cholesterol and reducing the

risk of chance of heart disease. Therefore, the total amount of phenolic compounds in *Gymnema sylvestre* leaf extract was determined. The results showed that all extracts contained phenolic compounds at significant levels, ranging from 47.84 ± 0.37 μg $ml^{-1}$ (80% ethanol extract) to 22.94 ± 0.19 μg $ml^{-1}$, suggesting that the antioxidant activity of the extracts was at least partially mediated by phenolic components. SOD present in the body is known to inhibit the reaction that converts active oxygen to hydrogen peroxide in cells. In addition, the consumption of foods rich in antioxidants has been recommended to prevent diseases caused by oxidation. Blood glucose levels in diabetic rats fed *Gymnema sylvestre* extract decreased to normal levels. The presence of the anti-hyperglycemic compounds gymnemagenin and gymnemia acid in the extract of *Gymnema sylvestre* was detected by LC/MS analysis. Lipid peroxidation levels were reduced by 31.7% in serum, 9.9% in liver and 9.1% in kidney in diabetic rats fed the extract. Five water-soluble polysaccharides, including GSP11 (xylose:glucose = 1:2.47), GSP22 (rhamnose:glucose: galactose = 1.6:1:1.22), GSP33 (rhamnose:glucose: galactose = 1:1.5:1.1), GSP44 (xylose: glucose: galactose = 1:1.03:1.2) and GSP55 (rhamnose:xylose:glucose: galactose = 1:1.21:1.81:2.58), were obtained from *Gymnema sylvestre* and tested for their immunological and anti-tumor activities. In this study, the anti-tumor activities of the seven polysaccharide samples (crude, GSP, GSP11, GSP22, GSP33, GSP44 and GSP55) were tested on Satellite glial cells, AGS and U937 cells. All seven polysaccharide samples inhibited the AGS, SGC and U937 cells, and the inhibition of tumor proliferation increased as their concentrations increased, except for GSP22 in the AGS cells. These results revealed that the crude, GSP, GSP44 and GSP55 samples had none or weak activity in AGS, SGC and U937 cells. GSP11 and GSP33 showed weak activities in AGS and SGC cells; however, their inhibitory rates in U937 cells were 78.6% and 83.8%, respectively, at a concentration of 640 μg $ml^{-1}$. GSP22 showed weak activity in AGS and U937 cells; however, the inhibitory rate of GSP22 (640 μg $ml^{-1}$) in SGC cells was 78.2% [34].

Chakraborty *et al.* [35] investigated whether *Gymnema sylvestre* has anticancer potential and, if so, to elucidate its possible mechanism of action. They initially tested the anticancer potential of *Gymnema sylvestre* on A375 (human skin melanoma) cells *via* MTT assay and determined the cytotoxicity in A375 and hepatocytes. normal; then thoroughly investigated its apoptotic effects on A375 cells through protocols such as Hoechst 33258, H2DCFDA and rhodamine 123 stain and performed ELISA assays for cytochrome c, caspase 3 and PARP. They determined the mRNA level expression of cytochrome c, caspase 3, Bcl2, Bax, PARP, ICAD and EGFR signaling genes by semi-quantitative reverse transcriptase polymerase chain reaction and performed western blot caspase analysis. 3 and PARP. By analyzing cell cycle events, it was determined that the accumulation of reactive oxygen species and measured the intensity of nexnin V-FITC/PI and rhodamine 123 by flow cytometry. Compared with both normal hepatocytes and untreated A375 cells, the mortality of *Gymnema Sylvestre*-treated A375 cells increased in a dose-dependent manner. In addition, *Gymnema Sylvestre* induced nuclear DNA fragmentation and showed increased levels of mRNA expression of genes involved in cytochrome c apoptotic signaling, caspase 3, PARP and Bax, and decreased expression levels of ICAD, EGFR and the anti-apoptotic gene Bcl2. Therefore, the MTT assay showed that *Gymnema Sylvestre* had a significant cytotoxic effect on A375 cells while normal hepatocytes (WRL-68) did not show such a significant cytotoxic effect in test tube. The percentages of programmed death in the population treated with *Gymnema Sylvestre* (150 μg $ml^{-1}$, 200 μg $ml^{-1}$, and 250 μg $ml^{-1}$ over 24 h) were 23.42%, 25.37%, and 31, respectively 89% for A375 cells compared with control cells. The overall results indicate that *Gymnema sylvestre* has a significant antitumor effect on A375 cells in addition to its reported antidiabetic effect, suggesting a potentially reduced use in patients with symptoms of both diseases [35].

Ethanol extracts of *Gymnema sylvestre* leaves exhibited marked antitumor-promoting activity in an *in vivo* two stage carcinogenesis test in mice using 7,12-dimethylbenz[*a*] anthracene as an initiator and 12-*O* tetradecanoylphorbol-13-acetate (TPA) as a promoter. The first tumor appeared at week 5 in the group treated with DMBA plus TPA and all 15 mice had tumors at week 13. In the group treated with DMBA plus TPA and ethanol extract of *Gymnema sylvestre* leaves, the first tumor appeared at week 9. The percentage of tumor-bearing mice treated with DMBA plus TPA and ethanol extract of *Gymnema sylvestre* leaves was 27% at

week 20. From the active fraction of the ethanol extract of the *Gymnema sylvestre* leaves, three triterpenoids were isolated and identified. This is the first report to find that ethanol extracts of *Gymnema sylvestre* leaves inhibit tumor promotion by TPA following initiation with DMBA in ICR mouse skin. These compounds were evaluated for their inhibitory effects on TPA-induced inflammation (1 $\mu$g/ear) in mice. The tested compounds showed marked anti-inflammatory effects, with a 50% inhibitory dose of 50-555 nmol/ear. These results demonstrate the efficacy to two triterpenes and triterpene glycosides in the components of *Gymnema sylvestre* leaves. In this study, the numerous triterpenes and their glycosides proved to be effective for preventing cancer [36]. Inhibitory effects of ethanol extracts of *Gymnema sylvestre* leaves on tumor promotion of skin papillomas by TPA in DMBA-initiated mice is depicted in Fig. 5. From one week after initiation with a single topical application of 50 $\mu$g of DMBA, 1 $\mu$g of TPA was applied twice weekly. Topical application of ethanol extract (1 mg) and vehicle was performed 30 min before each TPA treatment. Data are expressed as the percentage of mice bearing papillomas (a) and as the average number of papillomas permouse (b).e, +TPA with vehicle alone; I, +TPA with ethanol extract of *Gymnema sylvestre* leaves. The treated group was determined to be statistically different from the control group by Mann-Whitney $U$ exact test (a) and by student's $t$-test (b).

*Gymnema sylvestre leaves* extract against 7,12-dimethylbenz (a) anthracene (DMBA) induced papilloma genesis in Swiss albino mice was studied by Agrawal *et al.* [37]. The methanolic extract of *Gymnema sylvestre* was analyzed for chemo-preventive activity. Chemo-preventive activity was evaluated by two stage protocol consisting of initiation with a single topical application of a carcinogen (7,12-dimethylbenz (a) anthracene (DMBA) followed by a promoter (croton oil) two times in a week were employed. A significant reduction in tumor incidence, tumor burden and cumulative number of papillomas was observed, along with a significant increase in average latent period in mice treated tropically with *Gymnema sylvestre extract* as compared to the control group treated with DMBA and croton oil. The in vitro antioxidant activity of *Gymnema sylvestre* leaves was tested in various concentrations against Ascorbic acid as standard. Percentage of Thiobarbeturic Acid Reaction Substances (TBARS) was calculated for both Ascorbic acid and *Gymnema sylvestre* extract, with the help of formula, for a comparative experiment. The antioxidant and antibacterial effect of *Gymnema sylvestre* extract was also observed. As a result, In vitro antioxidant activities of *Gymnema sylvestre* extract showed significant inhibitory concentration as compared to ascorbic acid [37].

Pon Nivedha *et al.* [38] decided to design a study with the

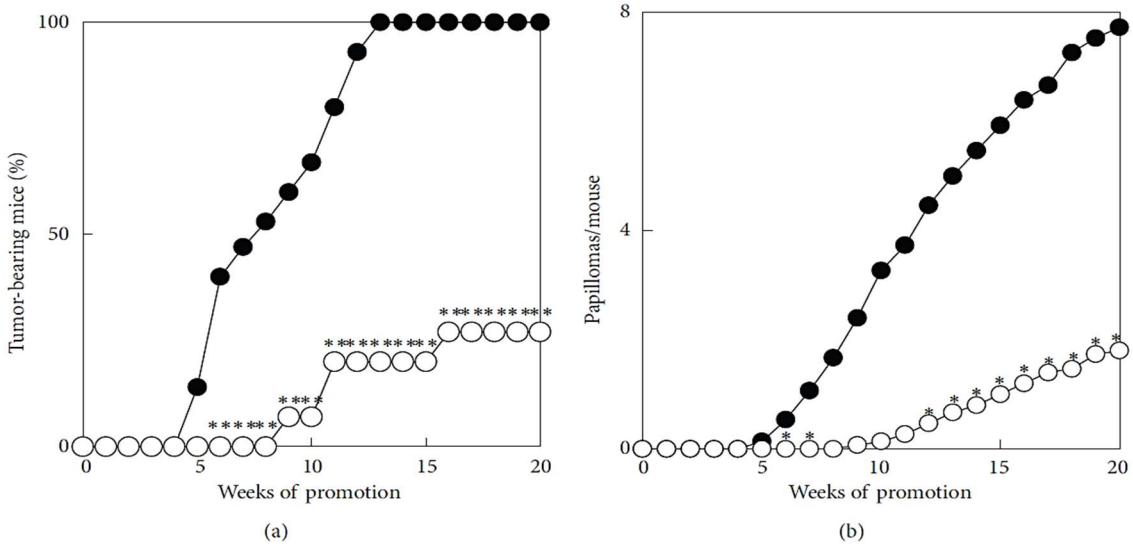


**Fig. 5.** Inhibitory effects of ethanol extracts of *Gymnema sylvestre* leaves on tumor promotion of skin papillomas by TPA in DMBA-initiated mice [36].

aim of producing chemo-preventing drugs. As prostate cancer is one of the frequently diagnosed cancers in men, quickly and effectively eliminate pre-malignant cells by inducing apoptosis. In this context, the study utilized an integrated approach of in silico and in vivo analysis to find an effective chemo-preventive drug, which induces apoptosis. Three phyto-compounds from *Gymnema sylvestre* were screened against anti-apoptotic proteins (Bcl-2 and Bcl-xl) through in silico analysis and the best compound was identified as dihydroxy gymnemic triacetate. Its chemo-preventive activity was analyzed in the N-methyl-N-nitrosourea and Testosterone-induced Sprague-Dawley rats. The result indicates that anti-apoptotic proteins (Bcl-2, Bcl-xl) level were significantly increased in cancer induced animals compared to control whereas treatment with dihydroxygymnemic triacetate supplementation significantly maintained the protein expression in par with control rats. Overall, this study suggests that dihydroxy gymnemic triacetate may act as a potential chemo-preventive agent in targeting the prostate cancer and it may open novel prospective in cancer chemoprevention [38].

In the study, done by Packialakshm and Sowndriya [39], leaf samples were exposed to four distinct solvents (ethanol, acetone, ethyl acetate, and distilled water), and the four extracts were then checked for phytochemicals using various biochemical tests. To examine the functional groups of AGS, Fourier-transform infrared (FT-IR) spectroscopy was used. Nitric oxide (NO) radical scavenging activity was used to gauge the antioxidant activity of AGS. The total antioxidant capacity of AGS was measured spectrometrically by phosphomolybdenum method, which is based on the reduction of Mo(IV) to Mo(V) by the analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm30. AGS exhibited different degrees of antioxidant capacity and the value was 23.07 mg AAE/g. The excellent antioxidant capacity might be due to the presence of phytochemicals present in AGS. Protein denaturation assay inhibition was used to study the anti-inflammatory activity, and MTT assay against MG63 cell lines was used to assess the anti-cancer activity. The presence of phytoconstituents like alkaloids, carbohydrates, triterpenoids, proteins, phenols, and flavonoids was confirmed by phytochemical screening. As it is shown in Fig. 6, to evaluate the cytotoxic effect of AGS, a MTT assay

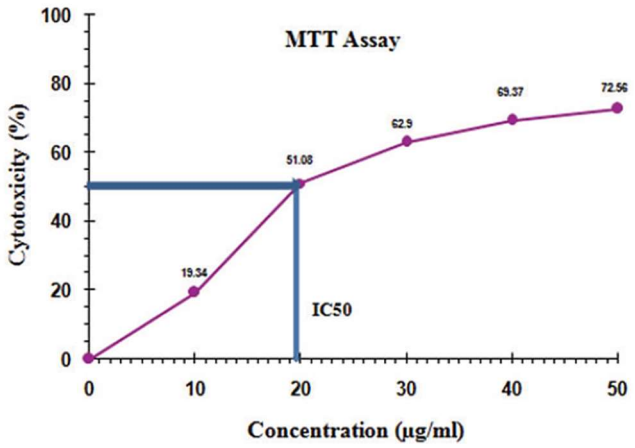


**Fig. 6**. Anticancer effect of AGS on MG63 cell line [39].

was performed on human osteosarcoma MG63 cell line. MG63 cell lines were treated with AGS at different concentrations (10 µg to 50 µg) and $IC_{50}$ (50% growth inhibition) value was determined. The cancer cell lines were inhibited significantly with the increasing of AGS concentration. A chart was plotted using the concentration of the AGS in X- axis and the percentage of cytotoxicity in Y-axis. AGS showed maximum cytotoxicity, 72.56% at 50 µg $ml^{-1}$ and the $IC_{50}$ value was found to be 19.5 µg $ml^{-1}$. Control did not show any cytotoxicity. The Limit of cytotoxic activity for the crude extract, are IC50 < 20 mg/ml38. The IC50 of AGS fall within the NCI guidelines limit, thus it has potential anticancer activity against MG63 cells. Preliminary phytochemical investigation revealed the presence of secondary metabolites in AGS. Therefore, the cytotoxic effect of AGS might be due to the presence of these secondary metabolites. Different peak values in the FT-IR spectrum indicated the presence of various functional compounds in AGS, and it demonstrated significant NO radical scavenging activity with an $EC_{50}$ value of 401.66 µg $ml^{-1}$ [39].

Antioxidant neutralizes hydroxyl radicals, super oxide radicals thereby proving its antioxidant nature and also showing blood sugar uptaking abilities. Terpenoid, Flavonoid, Cinnamic acid, Folic acid, Tannin, Phenol also scavenge SOD, $H_2O_2$ and having reducing power ability. Reduction of blood glucose and Glucose absorption in intestine. Stimulation of insulin secretion from β cell, glucose uptake and Inhibition of α-amylase and α-glucosidase. Both

antioxidant and antidiabetic function is very helpful in Type 2 diabetes treatment, and perform antidiabetic as well as antioxidant activity [40].

The study of Gosh *et al.* [41] was about evaluating the cytotoxicity potential of Gymnema sylvestre saponin rich fraction (GSSRF) on breast cancer cell lines (MCF-7 and MDA-MB-468) by SRB assay. The anti-tumor activity of GSSRF was assessed in tumor-bearing Elrich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLA) mouse models. The method to assess the anti-oxidant potential of GSSRF was DPPH radical scavenging assay. The in vitro cytotoxic effects of GSSRF on breast cancer cell lines were promising and found to be dose-dependent. An acute toxicity study of GSSRF was found to be safe at 2000 mg/kg body weight. GSSRF treatment has shown a significant increase in the body weight and the life span of EAC-bearing mice in a dose-dependent manner when compared with the control group. Figure 7 confirms the tumor volume in the control animals was significantly ($p < 0.05$) increased, with a maximum (0.85 + 0.01), on day 30 when compared with day 0. The treatment with GSSRF at 100 and 200 mg/kg significantly ($p < 0.05$) reduced the tumor volume (0.51 + 0.01) and (0.48 + 0.01) when compared with the control; a more significant reduction was seen at the dose of 200 mg/kg. The standard cisplatin reduced the tumor volume (0.42 + 0.01) in comparison with the control. In the solid tumor model, the doses of 100 and 200 mg/kg body weight per day have shown about 46.70% and 60.80% reduction in tumor weight and controlled the tumor weight until the 30th day when compared with the control group. The activity of GSSRF in both models was similar to the cisplatin, a standard anticancer agent used in the study. Together, these results open the door for detailed investigations of anti-tumor potentials of GSSRF in specific tumor models, mechanistic studies and clinical trials leading to promising novel therapeutics for cancer therapy [41]. Figure 8 shows the effect of GSSRF on DLA-induced solid tumor model (tumor weight).

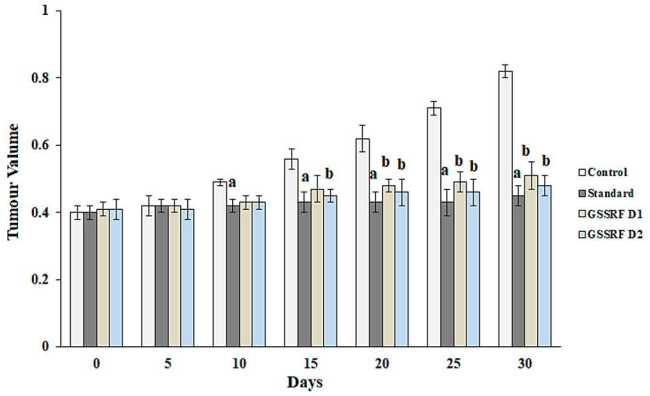


**Fig. 7.** Effect of GSSRF on DLA-induced solid tumor model (tumor volume) [41].

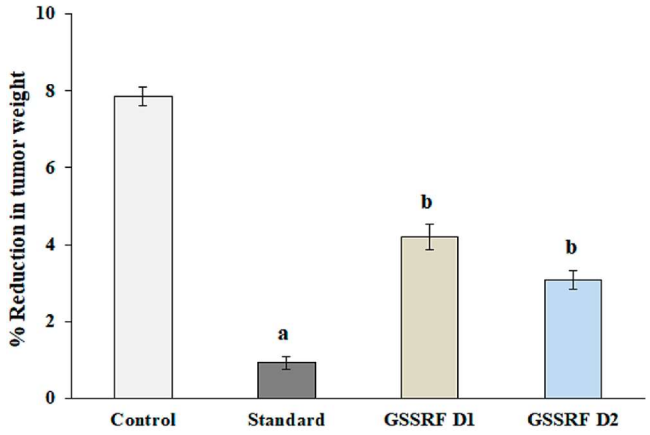


**Fig. 8.** Effect of GSSRF on DLA-induced solid tumor model (tumor weight) [41]

## CONCLUSIONS

In conclusion, the results obtained in the present study indicated the presence of alkaloids, carbohydrates, triterpenoids, proteins, phenols and flavonoids in the aqueous extract of *Gymnema sylvestre*. The extract effectively scavenged the nitrite radical and also showed potent anti-cancer and anti-oxidant activity against several human cancer cells. These activities may be due to the strong occurrence of polyphenolic compounds. We can conclude from various literature reviews that bio-components possess both cytotoxicity and antioxidant activities. As cancer incidence increases day by day, more effective research and assessment or analysis must be required to find out other active phytochemical compounds with exact active anticancer mechanism of action in near future. Therefore, the present review gives on idea that this extract can be used to design a potent anti-oxidant and anti-cancer drug.

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