ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

The cytotoxic effect of *Synadenium grantii* extract against human lung carcinoma A549 cells and its role in improvement of histopathological and biomarkers changes in Benzo(a)pyrene-induced lung cancer in rats

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Article History:

Received on: 20 Jun 2020
Revised on: 15 Jul 2020
Accepted on: 09 Sep 2020

Keywords:

Synadenium grantii,
Lung cancer,
Cytotoxicity,
Phytochemical screening,
Alpha-fetoprotein,
Phosphotylinositol 3 kinase,
Lung histology

ABSTRACT

Lung cancer is one of the most lethal cancers which is causing up to 3 million deaths annually worldwide. Therefore, management of lung cancer needs searching for new chemopreventive agents. This work was designed to inspect the chemopreventive potential of different extracts prepared from branches and leaves of *Synadenium grantii* for screening their effects on lung cancer cells (A549), then the most active extract was used for combating lung cancer induced in animal model. The *in vitro* results showed that, the methanolic extract was the most active extract against A549 cells with a notable cytotoxicity activity (IC_{50}: 4.30±0.44 μg/ml), which was close to the activity of standard drug, doxorubicin (IC_{50}: 3.50±0.40 μg/ml). The results of the *in vivo* experiment, revealed that in B(a)P-treated group, aspartate (AST) and alanine (ALT) transaminase activities as well as the levels of urea, creatinine, alpha-fetoprotein (AFP) and Phosphotylinositol 3 Kinase (PI3K) were significantly increased comparing to control group. However, treatment with *S. grantii* ameliorated the increase in these parameters in both after- and before-treatment groups comparing with B(a)P-treated group. This improvement in biochemical results were also supported by improving in morphological and histopathological injuries induced by B(a)P, which indicated that methanolic extract of *S. grantii* has a chemoprevention effect on lung cancer.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4230

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INTRODUCTION

New cancer cases are predicted to rise from 14 million in 2012 reach in 2035 to 24 million worldwide. While the greatest increments will be in poorest nations, where cancer services are hard pressed, even the richest nations will struggle to meet requests of increasing the number patients and providing the costs of treatment (Stewart *et al.*, 2016). Cancer is a common term for a large category of diseases that can affect any part of the body. Although the estimated number of cancer related deaths declined, cancer is still the main

cause of death (Mahmoud *et al.*, 2011; Abdullaziz *et al.*, 2017).

Lung cancer is the most occurring malignant disease. 1.8 million new cases of lung cancer were estimated in 2012, representing about 13% of from all total cancer detected (Sun *et al.*, 2019). It is also responsible for nearly 20% of all cancer deaths around the world (1.59 million deaths, 19.4% of the total) (Sun *et al.*, 2019). In Egypt, there is not an accurate epidemiological database about lung cancer because of lacking a national comprehensive record for population-based cancer. Otherwise, official statistics, hospital and institution studies revealed that this type of cancer is the second common cancer in men and also the second main cause of cancer mortality in Egypt after bladder cancer (Ibrahim *et al.*, 2013).

Herbs have been considered natural and valuable sources for anticancer drug discovery. Interest in ethnopharmacy has increased worldwide as a source of active compounds have pharmacological applications, particularly in the search of new treatments to cure of diseases (Marzouk *et al.*, 2009; Hussien *et al.*, 2019). In this context, the natural anticancer compounds extracted from plants may be used for human consumption and as a nutritional supplementation. A large number of plants are used as to overcome the various side effects of cancer (Abdel-Halim *et al.*, 2020).

Literature survey showed that many plants from Euphorbiaceae family have been used for various medicinal uses and having a number of health benefits. Otherwise, many of these plants are of significant economic values. Eminent members of this family include castor oil plant , cassava, Para rubber tree and Barbados nut. Numerous plants are serving as ornament, such as Poinsettia. In medicine, some species of Euphorbiaceae demonstrated active role in treatment genital herpes (Betancur-Galvis *et al.*, 2002). Also, Acalypha genus (Euphorbiaceae) containing many plants that traditionally used in various medicinal applications such as jaundice, fever, hypertension, diabetes, liver inflammation, schistosomiasis, dysentery, respiratory problems, skin conditions, antimicrobial, hepatoprotective, anticancer, leishmanicidal, anti-venom, analgesic, anthelmintic, antiemetic, laxative and diuretic (Seebaluck *et al.*, 2015). Among all these plants, *S. grantii* or Africa Milk Bush which was used in traditional medicine in peptic ulcers and inflammatory diseases. Also, *S. grantii* has been used as anti-bacterial, anti-oxidant, fibrinolytic agent and immunoregulator (Munhoz *et al.*, 2014).

The aim of the current work was targeted to prepare from *S. grantii's* stem several extracts using different solvents (water, methanol, ethanol and chloroform), then screening their cytotoxicity and anti-cancer activities on lung cancer (A549) cell line. Then the highly efficient extract was subsequently used to estimate its effect against lung cancer induced by B(a)p in rats.

MATERIALS AND METHODS

Chemicals

Dulbecco's modified eagle's medium was obtained from Cambrex (Biowhittaker, Switzerland). Benzo(a)pyrene, dimethyl sulfoxide, doxorubicin, penicillin, streptomycin, heat-inactivated fetal calf serum and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich chemical Co. (Deisenhofer, Germany). All other chemicals used were of the highest purity and analytical grade.

Plant materials and extracts preparation

S. grantii was collected from the Orman Botanical Garden, Giza, Egypt, under the supervision of Senior Agronomist Therese Labib of the Orman botanical garden. A full identification and authentication of this plant was carried out by Dr. Loutfy Bolous, Professor of Plant Taxonomy, Alexandria University. Botanical voucher specimens (B- 1475) were deposited at Herbarium of pharmacognosy department, National Research Centre, Egypt.

Green branches and leaves of *S. grantii* were dried at 40°C for one week in a solar oven and then powdered by using freeze dryer. Powder was stored in a dark place at 4°C for the subsequent usage.

About 400 g of powder were transferred to flasks and kept in dark places, then soaked in 1 L of solvents (water, methanol, ethanol and chloroform) and kept at room temperature with stirring for 48 h. The extracts were filtered and the sediments were re-extracted with the same solvent for 24 h. This process was repeated then the filtrates were combined and evaporated under reduced pressure at 40°C using Büchi Rotary Evaporator R-114 (Switzerland). The filtrate was dried using a Snijders Freeze Dryer (Holland) then the yield of each extract was calculated. The obtained powder was stored in sterile vials at -20°C (Hussien *et al.*, 2019).

In Vitro cytotoxicity study

In Vitro cytotoxic activity of various extracts was evaluated versus human lung A549 cancer cells. The cells were purchased from the American Type Culture Collection (Rockville, MD, USA), using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide] colorimetric assay procedure according to method of Abd-Elzaher *et al.* (2010); Rashad *et al.* (2012). Briefly, cells (10^4 cells/ well) were inoculated for 24 h in 96-well microtiter plate. Tested extracts and doxorubicin, as standard reference drug (widely used in treatment of various cancer diseases) were dissolved in dimethyl sulfoxide (DMSO) at 1 mg/ml then added to the cancer cells after diluting to appropriate volumes (3 wells were prepared for each individual dose). Cells were incubated in 5% CO_2 atmosphere at 37°C for 48 h. Then the cells were fixed, washed, and stained with MTT dissolved in DMSO 0.4% (w/v) for 30 min. Unbound dye was washed with DMSO. The intensity of color was measured in an ELISA reader (Asys Hitech, Austria) at 570 nm. The concentration of the extract required for 50% inhibition of viability of the cancer cells was calculated as IC_{50} (El-Malah *et al.*, 2016).

Phytochemical screening of *S. grantii* methanolic extract

The methanolic extract of *S. grantii*, which gave the best IC_{50} value on the lung cancer cells, was used for the subsequent phytochemical analysis which include qualitative evaluation of alkaloids, terpenoids, saponins, flavonoids, phenols & tannins, carbohydrates & steroids according to (Hussien *et al.*, 2019).

Animals

Male albino western rats (body weight of 130 ± 25 g) were purchased from the animal house of National Research Centre (Egypt). During the experimental period, rats were stayed under standard laboratory conditions in a room at with temperature 24±3°C and 12 h light/dark cycles. Rats were supplied with tap water and commercial diets. The experimental procedures used in this study were carried out in accordance with Institutional Ethical Guidelines for the care of laboratory animals of the National Research Centre, Egypt (Registration Number: 15/117).

Median lethal dose (LD_{50}) determination

LD_{50} of methanolic extract of *S. grantii* was evaluated according to Ali *et al.* (2018). Doses of *S. grantii* methyl extract up to 4000 mg /kg body weight dissolved in DMSO were administered to the rats orally (6 rats in each group).

After 24 h, the toxic indicators and death rate were recorded in each group and the mortality was observed up to one month post-treatment. The LD_{50} of *S. grantii* methanolic extract was determined and 1/10 of the LD_{50} as a safe dose will be used throughout the study.

In Vivo experimental design

After acclimatization of the rats for one week, the animals were divided into 6 groups, 10 rats in each group. Control group in which rats were administrated the vehicle (saline) only. B(a)P-treated group where lung cancer was stimulated in animals by intraperitoneal injection of rats with 100 mg B(a)P/kg b.w. for 32 week (Ibrahim *et al.*, 2013). The methanolic extract group in which animals were administrated with 1/10 of the LD_{50} of extract daily by oral gavage using an intragastric tube for 30 day, then the animals were given the vehicle through the experiment. After-treated group in where the animals were gavaged with the methanolic extract after cancer development. Before-treated group where animals were gavaged with the extract before the injection with B(a)P and then the animals were given the vehicle till the end of the experiment.

At the end of the experiment (32 week), All the animals were weighed, anesthetized, blood was withdrawn from the retro-orbital plexus and kept at room temperature to allowed to clot, then sera were collected by centrifugation at 3000 rpm for 15 minutes and then sera were aliquoted and stored at -20°C to be used for subsequent analysis. Moreover, the rats were dissected then the lobes of the lung were excised and photographed while other part of the lung were used for the histopathological examination.

Biochemical analysis

The activities of serum ALT and AST as liver function test were determined spectrophotometrically using reagent kits obtained from Biomerieux (France). Also, serum urea and creatinine levels as kidney function test were estimated by using kits supplied by Chornolab (Barcelone, Spain). In addition, serum AFP and PI3K concentrations as tumor markers were determined using ELISA kit obtained from Immunospec Corporation, USA.

Histopathological study

Biopsies from lung tissues were fixed in 10% formalin, embedded in paraffin, then samples were processed to obtain 5 μm thickness by microtome. Lung sections were collected on slides, deparaffinized and stained with hematoxylin and eosin (H&E). The slides were examined by a Leica photomicroscope.

Statistical analysis

Data were presented as mean ± SE. Statistical differences between different treatments were calculated by one-way analysis of variance (ANOVA) by using SPSS, version 9.0 followed by least significant difference test to detect differences between groups. Significance level was set at $p < 0.005$.

RESULTS AND DISCUSSION

In Vitro cytotoxicity assay

The preliminary study using various extracts of *S. grantii* (water, methanol, ethanol and chloroform) on A549 cell line revealed that water, ethanol, chloroform extracts have little effect with IC_{50} value 90.15±2.50, 125.27±2.00 and 197.66±2.40 μg/ml respectively, while the crude methanolic *S. grantii* extract was the most active extract against A549 cell line and it has IC_{50} 4.30±0.44 μg/ml that was so closed to the positive control, doxorubicin (IC_{50} 3.50±0.40 μg/ml), so the methanolic extract was used to investigate its anticancer effect throughout the *in vivo* study (Figure 1).

Phytochemical screening of *S. grantii* methanolic extract

The data of the phytochemical screening is presented in Table 1. The results showed presence of high flavonoids concentration. While, phenols; tannins; terpenoids; steroids; carbohydrates and alkaloids present in moderate concentrations. Whereas, saponins were found in low concentration.

In Vivo determination of LD_{50}

The result for the determination of the extract save dose revealed that LD_{50} of the methanolic extract was found to be 3050 mg/kg b.w. So, the oral dose of extract which was given to the experimental animals throughout the experiment was 305 mg/kg b.w., which represents 1/10 of LD_{50}.

Biochemical analysis

Data in Figure 2 demonstrated that lung cancer induced by (a)P resulted in a significant increment in serum ALT and AST activities comparing with control group. Data are expressed as mean values ± SE. a and b is significant difference from control and B(a)P- treated groups respectively at $p < 0.05$. However, treatment with the extract in after and before-treated groups resulted in significant decrease in their activities compared with B(a)P group. Moreover, the administration of extract only showed no marked effect on the previous enzymes in comparison to control group.

Otherwise, data presented in Figure 3 showed the statistical results of urea and creatinine levels in various experimental groups. Data are expressed as mean values ± SE. a and b are significant difference from control and B(a)P- treated groups respectively at $p < 0.05$. The current study demonstrate a highly significant elevation in the level of urea and creatinine ($p<0.0001$) in group treated with B(a)P as compared to control. The levels of both urea and creatinine were significant decrease in the after- and before treated groups compared to (a)P group. Moreover, the results revealed that the administration of extract alone had no marked impact in comparison to control group.

AFP concentration was measured in the serum of various experimental groups. Statistical analysis for AFP is shown in Figure 4,a and Figure 4 b are significant difference from control and B(a)P- treated groups respectively at $p < 0.05$. The data in this study showed significantly ($P<0.0001$) increase by 7- fold in the level of serum AFP in the group injected with B(a)P comparing with control. While there is a significant decrease in AFP level in both the pre- and after- treated groups compared to B(a)P group. On the other hand, rats gavaged with extract only showed non-significant changes in serum AFP level comparing to control group.

Measuring of serum PI3K level in various experimental groups and their mean values ± SE are shown in Figure 5, a and b are significant difference from control and B(a)P- treated groups respectively at $p < 0.05$. The results revealed that PI3K level was significantly increased in B(a)P group as compared to control group with a significant difference ($p<0.0001$), While the administration of extract, before and after treatment with B(a)P was noticeably decreased PI3K level as compared to B(a)P. While, rats gavaged with extract only showed non-significant changes.

Morphological examination of the lung tissues

The morphological investigation of lung tissues taken from different studied groups was illustrated in Figure 6. Where: (A) Control group, (B) B(a)P group, (C) Extract group, (D) Pre-treated group and (E) Post-treated group. It was demonstrated from the results that the lung of control group showed normal morphology with no nodules (Figure 6A). In B(a)P group, there was enlarged lung in size and a large number of buss nodules were scattered on the peripheral surface of the lung (Figure 6B). Also, the treatment with extract group only showed normal lung morphology (Figure 6C). A significant reduction in the number of the nodules was showed in after- and before-treated groups in comparison to group treated with B(a)P (Figure 6 D and Figure 6 E).

Histopathological evaluation of lung tissues

The histopathological investigation of lung tissues from various groups was shown in Figure 7 , where: (a) Control group, (b) Extract group, (c and d) B(a)P group, (e) Pre-treated group and (f) Post-treated group (H&E., x 400). Lung tissues from control group revealed normal architecture of the lung tis-

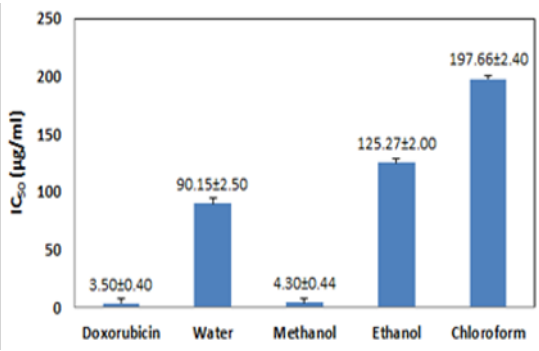

Figure 1: Cytotoxic activity(IC_{50}, μg/ml) of the different extracts of *S. grantii* extract against A549 cell line

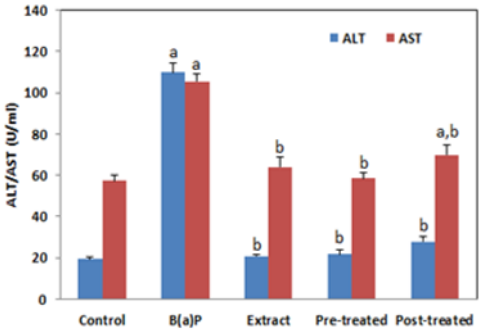

Figure 2: Serum ALT and AST activities in different groups

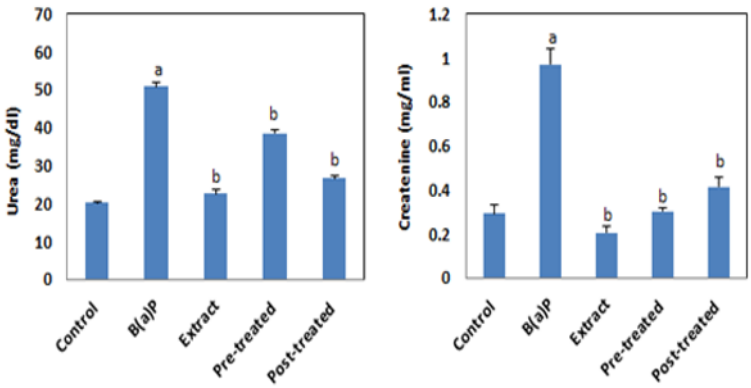

Figure 3: Serum urea and creatinine levels in different groups

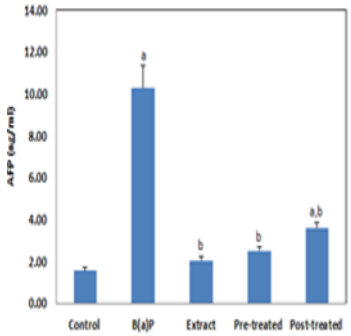

Figure 4: Mean values ± SE of serum AFP concentration (ng/ml) in different groups

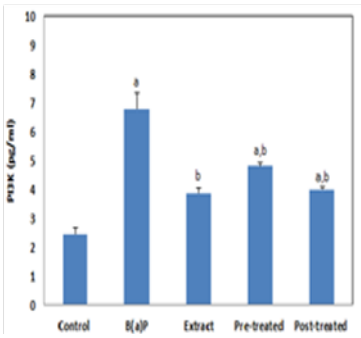

Figure 5: Mean values ± SE of serum PI3K level (pg/ml) in different groups

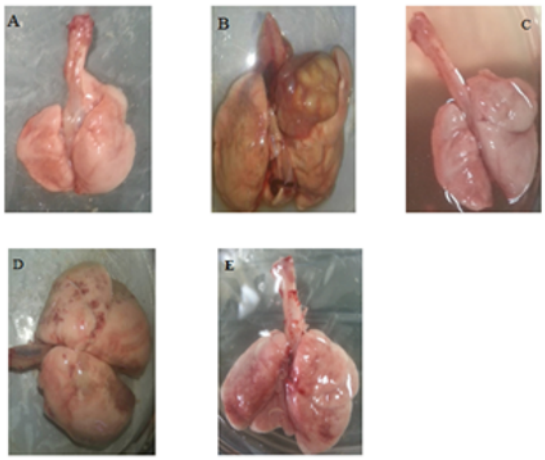

Figure 6: Macroscopic appearance of rat lung tissues from different studied groups

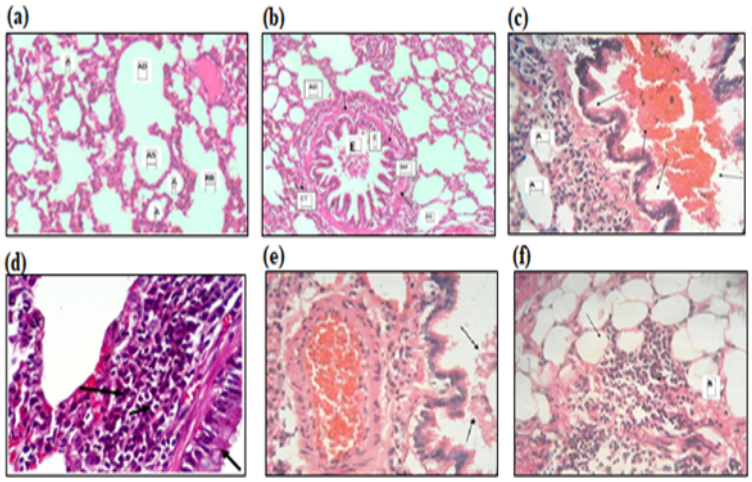

Figure 7: Photomicrograph sections of lung tissues from various groups

sue with normal bronchial tree (bronchus, bronchioles, alveolar ducts and alveoli) and inter-alveolar septa, respiratory bronchiole (RB), alveolar ducts (AD), alveolar sacs (AS) and individual alveoli (A). Bronchioles consist of mucosa as well as muscle and a connective tissue layer. Mucosa was appeared as a simple columnar ciliated epithelium with goblet cells. The epithelium rests on a lamina propria, a continuous well-developed muscle layer and a connective tissue thin layer (Figure 7a). Similarly, tissue from extract treated group showed no pathological abnormalities with normal alveolar duct (AD) and sacs (AS) and a large bronchiole has the characteristically folded respiratory epithelium (E) and prominent smooth muscles, but it is supported by fibrous connective tissues (CT) (Figure 7b).

Sections from B(a)P group revealed severe degenerative, diffuse alveolar damage. Some alveoli are collapsed, while others are distended with many are lined with hyaline membranes (arrows) (Figure 7c). In addition, there are basal cell hyperplasia, Papil-

Table 1: Phytochemical screening of *S. grantii* methanolic extract

Constituents	Methanolic extract
Flavonoids	+++
Phenols & Tannins	++
Terpenoids	++
Steroids	++
Carbohydrates	++
Alkaloids	++
Saponins	+

+ Weak reaction, ++Moderate reaction, +++ Strong reaction

lary hyperplasia of the epithelial cells in alveoli and bronchi, squamous metaplasia, various grades of dysplasia, nuclear hyperchromasia in the bronchial epithelium, infiltration of the alveolar septa with mononuclear cells containing darkly stained nuclei, well characteristic squamous cell carcinoma (Non-small lung carcinoma) (arrows) (Figure 7d).

Sections from rats in the pre-treated group showed a healing stage is marked by resorption of hyaline membrane with the thickening of the alveolar septa containing inflammatory cells, fibroblasts and collagen (arrows) (Figure 7e).

Tissues from rats of post-treated group showed low degenerative changes in the bronchiolar epithelium with low damage in the endothelial cells which indicate improvement of the pulmonary lesions, areas of large irregular air space (B) where only inflammation ranging from bronchitis to interstitial pneumonitis was appeared with reduction in mononuclear cellular infiltration of the alveolar septa with dark stained nuclei (arrows) (Figure 7f).

The requirement for the discovery of novel chemotherapeutic agent is needed for the prevention and treatment of cancer and to avoid the adverse effects of the chemotherapeutic drugs and their resistance (Ali *et al.*, 2009). Due to the little toxicity and high efficacy of the natural products so it popular used broadly in treatment protocol of patients with advanced cancer as complementary and alternative medicines (Hussien *et al.*, 2019).

S. grantii is one of the plants which belongs to the family *Euphorbiaceae*, which had been reported to have some pharmacological properties. Latex from *S. grantii* is commonly used in folk medicine to treat allergies and gastric disorders. In a previous study on the stem bark from *S. grantii* in both *in vitro* and *in vivo* assays, it demonstrated a significant antioxidant and anti-inflammatory activities, which were related to high content of phenolic compounds and terpenes compounds (Campos *et al.*, 2016).

The principal goal of the present work is to prepare various extracts (water, methanol, ethanol, and chloroform) from *S. grantii* as a natural substance and screening their effect on lung cancer cell line and its ability to combat tumorigenesis of lung cancer animal's model.

In the present work the potential cytotoxicity of different extracts from *S. grantii* against lung cancer cell line (A549) was evaluated. From the results, it was clear that methanolic extract of *S. grantii* had a notable cytotoxicity activity against A549 cells more than the other extracts and its activity against cancer cells was near to the cytotoxicity of standard drug, doxorubicin. The cytotoxicity activity of the methanolic extract can be attributed to the presence of high concentration of flavonoids leads to anti-proliferative activity of this extract as mentioned in the phytochemical screening (Table 1).

Consistent with our results, *de Oliveira et al. (2013)* isolated a glycoprotein from *S. grantii* and they found that this isolate exert its anti-tumor activity via inhibition of the tumor cells proliferation and induction of apoptosis of A-549 cells through suppression of NF-k activation as well as induction of c-Jun/Fra-2 dimerization. However, its antitumoral properties have been demonstrated to produce *in vitro* cytotoxicity (Baloch *et al.*, 2008).

Moreover, Dasari *et al.* (2012) explained that the presence of phenolic compounds of *S. grantii* is responsible for its ability to scavenge free radicals and to its ability to downregulate inflammatory response as used in folk medicine. Otherwise, *S. grantii* contains a large number of phenolic compounds, such as resveratrol, which affect many intracellular mediators and inhibiting lipid peroxidation more stronger than the antioxidants butylated hydroxyanisole, trolox and α-tocopherol.

In the *in vivo* study, lung cancer was induced in animals by administration of B(a)P, which is a very effective carcinogen promoting oxidative stress and consequently aiding in induction of free radical formation which in turn react with lipids in the membrane of the cell causing lipid peroxidation (Deng *et al.*, 2018). The current study showed a highly significant elevation in the activities of serum AST and ALT enzymes in B(a)P group in comparison to the control, indicating that B(a)P could induce a liver damage in the rats. This increase is due to the leakage of the enzymes from the damaged hepatocytes to the blood stream (Rašković *et al.*, 2014) . In cancer conditions, the disturbance in the transport function of hepatocytes is resulting in the alteration in the permeability of plasma membrane and thus causing an increased level of these enzymes in serum and

decreased their level inside the cells. Other explanation has demonstrated that hepatic metabolism of nitroso compounds; especially B(a)P produces reactive oxygen species that cause oxidative stress and substantial cellular injury, giving an additional support of the changed liver function enzyme activities (Ibrahim *et al.*, 2013).

On the other hand, treatment with *S. grantii* decreased the activities of these enzymes in after-, and before-treated groups in comparison with B(a)P group. These results suggested that *S. grantii* may have potential protective and therapeutic effects as well as safety against B(a)P. Administration of *S. grantii* only had no marked effect on the previous enzymes, reflecting the safety of the extract. These results were established the role of *S. grantii* methanolic extract in lowering the severity of cancerous alteration.

The current study showed that, serum urea and creatinine levels were significantly increased in tumorized rats. while, in after-, and before-treated groups their levels were significantly reduced comparing with B(a)P group. Overall, it can be postulated that there are no untoward effects of the methanolic extract on both liver and kidney. Therefore, the methanolic extract can be used without any serious effect.

The tumor markers AFP and PI3K were measured as indicators of lung carcinoma incidence in the serum of experimental animals. The data showed significantly increased in serum AFP and PI3K levels in the B(a)P group by (7- fold) and (4-fold) respectively as compared to control group. Whereas, treatment with the extract either after or before tumor induction was able to normalize their levels when compared to B(a)P group. This reduction in AFP and PI3K level with *S. grantii* revealed the antitumor effect of *S. grantii* extract. Otherwise, the improvement effect of *S. grantii* on AFP and PI3K in before-treated group was more pronounced than that in after-treated group. It is noteworthy that rats treated with *S. grantii* alone showed non-significant changes in both AFP and PI3K levels, suggesting the great safety of this extract.

PI3K is a measured as a tumor marker in lung cancer as PI3K/Akt/mTOR pathway, that has a critical role on oncogenesis of cancer through regulating cell survival, growth and proliferation. Emerging evidence by Sun *et al.* (2015) suggested that PI3K/ AKT/mTOR signaling is frequently activated in Non-small-cell lung carcinoma and it plays important roles in the progression through promoting survival, growth, proliferation and migration of cancer cells.

In the present study the morphological and histopathological investigations were confirmed biochemical results. The morphological examination of different experimental studied group demonstrated that both control and *S. grantii*-treated groups showed normal lung morphology, while B(a)P-treated group showed enlarged lung in size and a large number of buss nodules were scattered on the peripheral surface of the lung. Treatment with *S. grantii* improved the morphological changes where the number of nodules reduced on the lung comparing to B(a)P- group, which indicated the antitumor effect of *S. grantii* on rats. The most improvement was noticed in before-treated group, where than the after-treated group.

These morphological findings were in agreement with the histopathological examination where, lung sections of control group showed normal cellular architecture appearing as normal bronchial tree (bronchus, bronchioles, alveolar ducts and alveoli) and inter-alveolar septa. *S. grantii*-treated group showed normal lung architecture where lung cells appeared as control group, revealing the non-toxicity of *S. grantii*. Sections of lung from B(a)P-treated group showed severe degenerative alterations in the bronchiolar epithelium, mononuclear cellular infiltration of the alveolar septa, with dark-stained nuclei. It was mentioned that B(a)P administration can induce different proliferative and neoplastic lesions in rat lung tissues.

On other hand, treatment with *S. grantii* improved the carcinogenic changes in before- and after-treated groups. The improvement in before-treated group was more effective than that in after-treated group, where, before-treated group showed the healing stage is marked by resorption of hyaline membrane with the thickening of the alveolar septa containing inflammatory cells, fibroblasts and collagen, as well as restoring of normal architecture. The after-treated group showed minor changes in the form of showing mononuclear cellular infiltration of the alveolar septa with dark-stained nuclei and areas of large irregular air space, but no dysplasia or malignancy was detected. So, treatment with *S. grantii* resulted in normalizing the histological changes produced by B(a)P.

CONCLUSIONS

Our study showed that, methanolic extract of *S. grantii* could be used as a promising chemopreventive agent against lung cancer. This action appeared through stopping tumorigenesis incidence, normalizing the biochemical parameters and improving the morphology and preventing the multiplicity of neo-

plastic nodules. Also, it had a protective effect rather (as shown in pre-treated group) than therapeutic effect (as shown in before-treated group) against B(a)P-induced lung carcinogenicity in rats. More future explanation and investigation is required to elucidate the detailed mechanism of action, also more future identification of potent constituents from *S. grantii* that cause its chemopreventive and anti-cancer effect.

ACKNOWLEDGEMENT

Thanks are due to National Research Centre, Egypt for the financial support (Grant Number: 15/2/11).

Funding Support

The authors declare that they have funding support from National Research Centre (Grant Number: 15/2/11).

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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