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Potency of *Moringa oleifera* leaf extract and silver nanoparticles against immune, microbial and HT-29 colon cancer cells growth modulation

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Abstract: *Moringa oleifera* plant grows in many countries worldwide and being utilized as a customary medication. The current study aimed to investigate the biological effect of *Moringa oleifera* leaf extract (MOE) alone or in combination with silver nanoparticles (AgNPs) on colon cancer, microbial cell growth. MOE was utilized in the green synthesis of AgNPs. The characterization of AgNPs was done by UV-Vis-spectrophotometry, X-ray diffraction (XRD) and scanning electron microscopy (SEM). MOE was tested for their sugars, active biomolecules, ROS, protein contents. Results revealed that created AgNPs are about 61 nm in diameter. There were no detectable sugar and protein in MOE, but it contains ROS and active biomolecules. MOE and MOE+AgNPs exerted mild antibacterial action and increased the number of apoptotic cells and p53 protein expression of HT-29 colon cancer cells. MOE and MOE+AgNPs could arrest HT-29 cells at G2/M phase and stimulate splenic cell growth. Both extract preparations showed antioxidant activities. Because MOE and MOE+AgNP stimulated immune cells and activated apoptosis in cancer cells, these preparations can be utilized as anticancer agents.

Keywords: HT-29 colon cancer cell, xell-cycle arrest, ROS, p53, apoptosis.

INTRODUCTION

Therapeutic plants can be discovered wherever on the planet. Medicinal plants, including their extracts, are promptly accessible, financially savvy and are utilized in drug creation (Khor *et al*., 2020; Madi *et al*., 2016). The utilization of therapeutic plants is considered as a useful source of biomolecules with medicinal potential that can apply various bio-signaling pathways (Madi *et al*., 2016). To yield an expected medicinal consequence they can work in synergism. In any case, more endeavors and examinations are needed to guarantee the safety, the quality and adequacy of these plants being utilized as substitute therapeutics. Therapeutic plants that are favorable for the utilization as antitumor agents may tackle the current issues such as resistance to the medications, expenses and results.

Moringa oleifera (member of the family Moringaceae), is a tree being utilized in the conventional medicine, and has great potentials in medication (Tiloke *et al*., 2016). *M. oleifera* is situated over the world including Saudi Arabia (Vergara-Jimenez *et al*., 2017; Wu *et al*., 2021). The

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majority of the plant parts are utilized as food and in the conventional treatment. It likewise shows antibacterial/ viral, high antioxidant content, antifungal, diuretic, anti-hyperglycemia, wound healing action, neuroprotective, anti-cancer, hepatoprotective, antihypertensive, hepato-protective, modulate the immune system and anti-inflammatory properties, cholesterol lowering activity, and cardioprotective (Matic *et al*., 2018). *M. oleifera* progresses the of liver and kidneys functions and controls the thyroid hormones (Vergara-Jimenez *et al*., 2017). The antioxidant activity is particularly strong in leaves (Wright *et al*., 2017). *M. oleifera* has a high contents of phytosterols (Gopalakrishnan *et al*., 2016) vitamins (Vergara-Jimenez *et al*., 2017), terpenoids, glycosides and anthraquinones (Gopalakrishnan *et al*., 2016).

Studies revealed that *M. oleifera* possesses some potentials for targeting many agents to induce cancer progression inhibition (Abd Karim *et al*., 2016). Cancer cells grow very fast, therefore anticancer factors are designed to interrupt this process. *M. oleifera* has anti-tumor capacity against several cancers (e.g. liver cancer cells, myeloid and acute lymphoblastic leukemia, and pancreatic, breast, and A549 lung cancer cells) (Vergara-Jimenez *et al*., 2017). The cell cycle can be interrupted by some agents (e.g. β-sitosterol-3-O-β-D-glucopyranoside, 4-α-L-rhamnosyloxy benzyl isothiocyanate, D-allose, eugenol, hexadeconoic acid ethyl ester, isopropyl isothiocyanate and) leading to accumulation of the cells into sub-G1 phase. Moreover, leaves of *M. oleifera* upregulates reactive oxygen species synthesis leading to stimulation of caspases and p53. Glucosinolates (an effective anti-cancer agent) present in Moringa can enhance apoptosis sequences (Abd Karim *et al*., 2016).

The innovation of metal nanoparticles (NPs) has improved modern medicine because of their uncommon attributes which impact their method of working (Silveira *et al*., 2018). Materials extracted from plants are utilized in green synthesis of NPs (act as reducing and capping agents) (Silveira *et al*., 2018). plant extracts are generally rich in bioactive molecules (e.g. polyphenols) which can convert metals into NPs (Silveira *et al*., 2018).

A large portion of the past research demonstrated that Moringa leaf extracts have the power to terminate tumors, yet didn't show the impacts on similar healthy cells (i.e. normal quick dividing cells). In our study, interestingly, the anticancer capability of Moringa leaf acetone extract on the fast dividing cancer cells (HT-29 colon cancer cells) were compared to the quick growing normal cell (growth-stimulated splenocytes). Likewise, other biological bio-properties of the extract such as antibacterial, oxidant/antioxidant, acute toxicity, cell cytotoxicity, apoptosis, cell cycle and nanoparticles formation and characterization were tested.

MATERIALS AND METHODS

Plant collection and extract preparation

During October 2020, a significant amount of *Moringa oleifera* (MO) leaves were harvested from the Al Soudah (18°16′19.2″N 42°22′19.2″E), Saudi Arabia. Acetone extract of these leaves (MOE, 10 mg/mL stock solution) was created the same way as described by Ibrahim *et al*. (Ibrahim *et al*., 2021) and kept at -31°C.

Preparation and characterization of silver nanoparticles

Synthesis of AgNPs utilizing MOE and its characterization were performed the same way as described by Ibrahim *et al*. (Ibrahim *et al*., 2021).

Extract's sugar, protein, ROS and Functional groups analysis

Functional groups, proteins and sugars (maltose, glucose, fructose, and sucrose) present in MOE and MOE with AgNPs (MOE+AgNP) were explored the same way as described by Ghramh *et al*. (Ghramh *et al*., 2020). Quantity of reactive oxygen species (ROS) in MOE was measured utilizing a Rat reactive oxygen species, ROS ELISA Kit (Cat#: E1924r, EIAAB) following the manufacturer's instructions.

Testing of antimicrobial power

The antimicrobial activity of MOE and MOE+AgNPs was tested utilizing Gram positive (*Staphylococcus aureus/Bacillus subtilis*) and Gram negative (*Proteus mirabilis/Escherichia coli*) pathogenic bacteria as described by Alsyaad and H Ibrahim (Al Syaad and Ibrahim, 2020).

In vitro cell culture testing

HT-29 human colon cancer cell line (Merk) maintenance and subculturing were performed following Ganesan *et al*. (Ganesan *et al*., 2020). Preparation of splenocytes and *in vitro* culture were performed following Ibrahim *et al*. (Ibrahim *et al*., 2019). Briefly, a spleen removed from a healthy adult male Sprague Dawley rat weighing 239 g, kindly supplied by animal house found at King Khalid University, was used to get splenocytes. Cells were adjusted to $0.4X10^5$/mL in culture medium. Study of the MOE and MOE+AgNPs cytotoxicity against HT-29 cancer cell line, Study of cytotoxic, proliferative and anti-proliferative effects of MOE and MOE+AgNPs against normal and stimulated splenocytes were done following the exact procedures shown by Ibrahim *et al*. (Ibrahim *et al*., 2021).

Quantification of p53 gene expression, apoptosis and flow cytometric analysis of cell cycle

Quantification of p53 gene expression, apoptosis and analysis of cell cycle in HT-29 cancer cells later the treatment of HT-29 cancer cell line with MOE and MOE+AgNPs were done following the exact methods described by Ibrahim *et al*. (Ibrahim *et al*., 2021).

In vivo studies

MOE and MOE+AgNPs were tested *in vivo* for acute toxicity (quantities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine), oxidant/antioxidant (measuring malondialdehyde, total antioxidant capacity, superoxide dismutase, Catalase, Glutathione in liver and kidney tissues) and carcinogenic effects (serum Arginase and Alpha-L-Fucosidase) according Al Syaad and Ibrahim (Al Syaad and Ibrahim, 2020).

Ethical approval

The study was performed in accordance with the guidelines put by the Ethical Committee of King Khalid University (HAPO-06-B-001).

STATISTICAL ANALYSIS

Experimental outcomes were calculated as means of 6 results ± SD. A Student's t-test (SPSS version 20) was done for all values. Values are considered as statistically significant when the *p* value is ≤ 0.05.

RESULTS

Production/characterization of silver nanoparticles

Silver nanoparticles formation was monitored through the change in color of the $AgNO_3$/ MOE mixture. After color changed (fig. 1B), the synthesis of AgNPs was checked spectrophotometrically (fig. 1D) and the results revealed the presence of specific peak of AgNPs at a range of 400-450 nm.

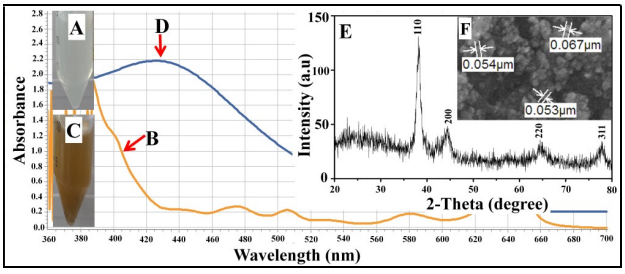

Fig. 1: Silver nanoparticles synthesis by *Moringa oleifera* leaf acetone extract and characterization. A: MOE; B: light absorbance of the MOE; C: MOE after adding of silver nitrate; D: light absorbance of MOE+AgNPs; E: XRD analysis of photosynthesized AgNPs and F: The SEM image showing AgNPs.

XRD study signifies that the NPs are crystalline and clear from (fig. 1E). There are about 4 major peaks in XRD profile situated at 38.19°, 44.31°, 64.73° and 78.44° which are resembles to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes, one-to-one as per standard data (JCPDS no: 4-0783) (Prakash *et al*., 2013). The d-spacing values are noticed as 2.359, 2.044, 1.445 and 1.231 Å for (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes. The size of crystalline (L) is intended by Scherrer formula, and found to be around 83 nm. SEM analysis uncovered that the shape of AgNPs are almost spherical with an average grain size of 61 nm (fig. 1F).

Functional groups

FTIR spectra of MOE earlier and later reduction of Ag are publicized in fig. 2. Vibrational bands in MOE formerly to bio-reduction are noticed in 500–3300 cm^{-1} range where strong bands appeared at 3290.6, 2908 and 2364 and are likened to O-H (alcohol), C-H (alkane) and O=C=O (carbon dioxide) stretching's. Strong bands at 1704- 1701.5 cm^{-1} is because of C=O of expanding aliphatic ketone. Powerful bands at 1597.2- 1519 cm^{-1} is allocated to C=C identical to aromatic compounds. Various bands 1311, 1010, 813, 762.2 and 520 cm^{-1} are related S=O (sulfone), C-O (alkyl aryl ether), bending C=C (trisubstituted alkene) vibrational modes and halo compound.

Absorbance bands in after bio-reduction of Ag ions revealed that transmittance of strong band of alcohols and medium band of alkane are markedly decreased, this might be owing to circumstance that they are mostly accountable for decrease of Ag ions. New weak bands arisen at 2329, 2000, 1554, 1449-1400, 1220-1137 cm^{-1} corresponding to stretching CΞC, N=C=S, C=C, bending C-H, C-O of alkyne, isothiocyanate, cyclic alkene, alkane and ester.

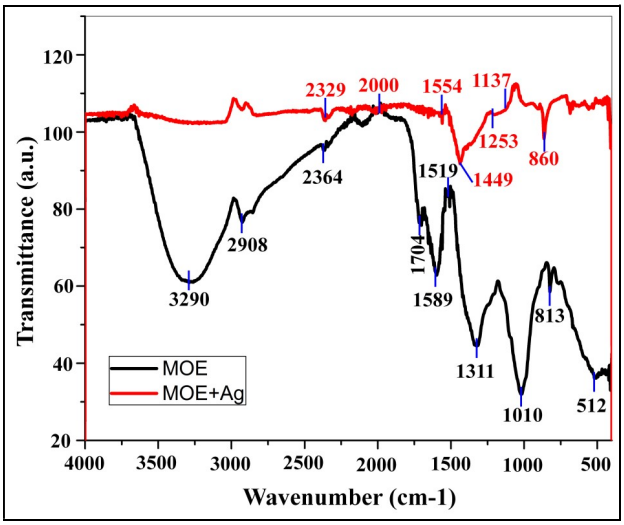

Fig. 2: FT-IR spectra of *M. oleifera* leaf extract (MOE) and MOE after the formation of AgNPs (MOE+Ag).

Sugar, protein and reactive oxygen species contents

Results showed that MOE contains a very minute amount of fructose, glucose, sucrose and maltose and no detectable protein fractions. The content of ROS is 158.3 pg/mL.

Antimicrobial potential

MOE and MOE+AgNP showed very weak antibacterial power to inhibit the growth all bacteria being tested.

Extract cytotoxicity against HT-29 cancer cell line

MOE showed a significant (p>0.0001) cell growth inhibitory effect on HT-29 cells at the concentrations 1000µg/mL down up to 125µg/mL while MOE+AgNPs showed a significant (p>0.0001) cell growth inhibitory effect at 1000µg/mL only. IC_{50} of MOE was shown to be at the concentration of 69.3 µg/mL and of MOE+AgNPs was 725µg/mL (table 1).

Effects on splenocytes

MOE showed a significant (0.0001) stimulatory effects on normal and stimulated splenocytes only at the concentration 1000 µg/mL (table 2). On the other side, MOE+AgNPs showed a significant (0.0001) stimulatory effects on normal and stimulated splenocytes only at the concentration 1000µg/mL. This stimulatory was significant (0.005) at lower concentration (500-7.8 µg/mL) with lower stimulation rate (table 2).

Table 1: Percent of HT-29 cell growth inhibition after treatment with MOE.

M. oleifera extract (µg/ml)	% of HT-29 cell growth inhibition	
	MOE	MOE+AgNPs
1000	88.93±0.21*	65.71±3.8*
500	82.57±0.921*	0
250	66.21±4.0*	0
125	53.79±5.6*	0
64	0	0
32	0	0
16	0	0
8	0	0
4	0	0
2	0	0
1	0	0
0.5	0	0

*= p= 0.0001

Table 2: Effects of MOE and MOE+AgNP on splenocytes growth rate.

Extract (µg/mL)	Growth rate of splenocytes (times over control)			
	MOE		MOE+AgNPs	
	Normal	PHA-stimulated	Normal	PHA-stimulated
1000	16.55±0.47	28.03±59*	20.64±0.50	30.32±0.62*
500	1.18±0.24	4.5±0.17**	1.02±0.21	3.65±0.18**
250	0.97±0.27	6.36±0.21**	0.95±0.12	6.61±0.24**
125	0.91±0.22	7.18±0.27**	0.99±0.13	8.63±0.39**
62.5	0.91±0.26	8.19±0.38**	0.95±0.89	9.19±0.32**
31.25	0.93±0.28	9.19±0.40**	0.81±0.81	9.74±0.38**
15	1.25±0.14	10.66±0.41**	1.34±0.14	11.46±0.36**
7.8	1.11±0.16	10.89±0.38**	1.18±0.18	12.54±.40**

*= p= 0.0001; **= p= 0.005

Quantification of p53 gene expression

We employed RT-PCR to quantitatively evaluate the countenance of p53 after treatment of human colon cancer HT-29 cell line with IC_{50} dose of MOE and MOE+AgNPs *in vitro*. Results demonstrated that IC_{50} dose of MOE and MOE+AgNPs increased p53 mRNA expression 3.68 and 7.58 folds over control cells respectively.

Cell cycle and apoptosis

Cell cycle arrest and apoptosis due to the effect of MOE and MOE+AgNPs preparations were investigated. Treatment of HT-29 cell line with MOE significantly (p>0.001) increased percent cell number in the G2/M population from 18.4 % (untreated control, 3E) to 25.2% (MOE treated, fig. 3A). Also, MOE significantly (p<0.05) increased the cells to go through apoptosis 7.83 % (3B) over control untreated cells (3F). In case of MOE+AgNPs treatment (3C), the G2/M population significantly (p>0.001) increased up to 47.9% when compared to the same control (fig. 3E). MOE+AgNPs significantly (p<0.05) increased apoptosis (29.45%, fig 3D) over the untreated cells (fig 3F). The decrease in percent cell growth is referred to apoptosis rather than necrosis as necrotic cells were shown to be at normal level in MOE treated cells (1.46%) and MOE+AgNPs treated cells (2.18%) which are nearly similar to untreated cells (1.19%).

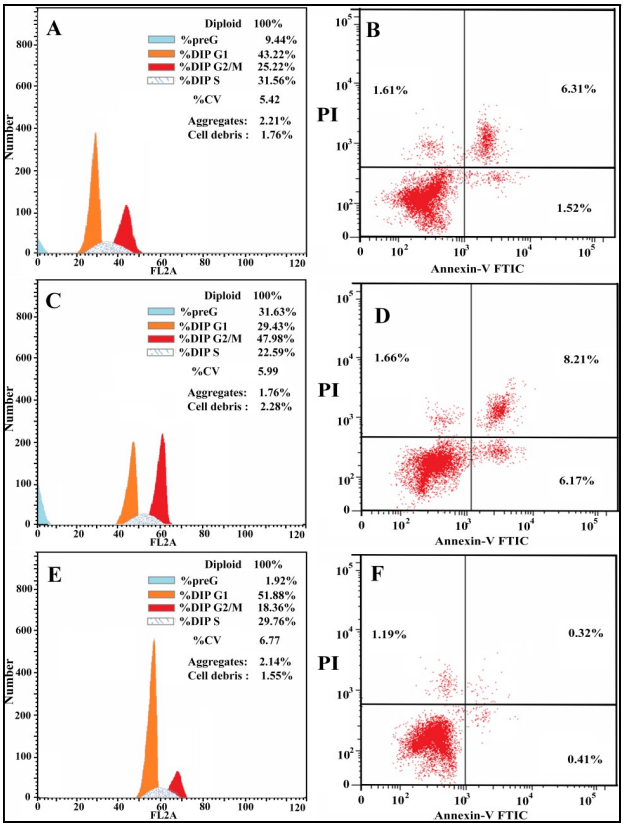

Fig. 3: Analysis of cell cycle progression (A, C and E) and apoptosis (B, D and F). Human colon cancer HT-29 cells were treated with MOE (A & B), treated with MOE+AgNPs (C & D) or untreated (E & F).

Antioxidants activities

Level of MDA showed a significant increase (<0.001) in liver tissues after treatment with MOE and MOE+AgNPs over the control groups and non-significant changes in kidney tissue (fig. 4A).

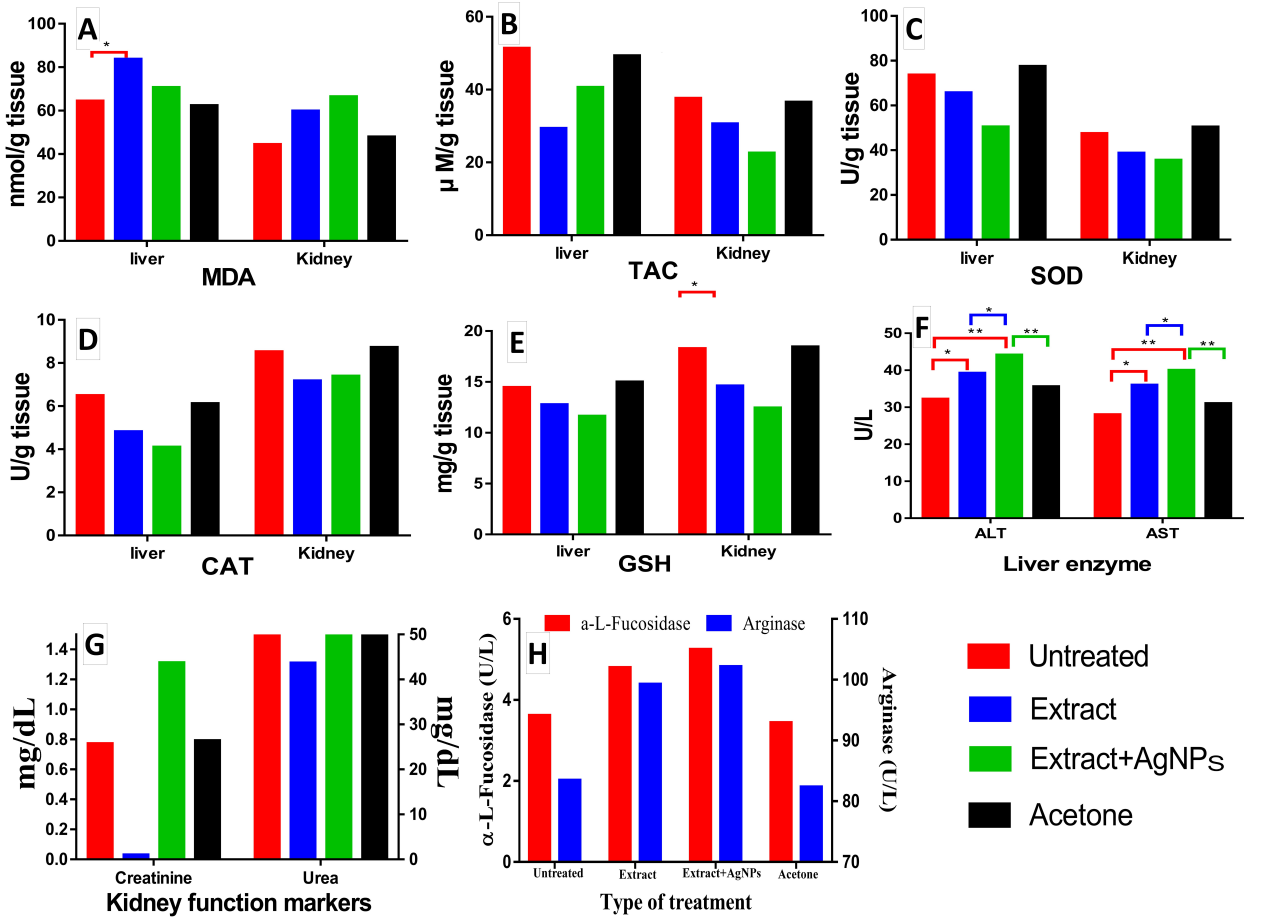

Fig. 4: Content of MDA (A), TAC (B), SOD (C), CAT (D) and GSH (E) in liver and kidney tissues of different groups; G: levels of AST and ALT; H: urea and creatinine in different treated groups and F: levels of serum Arginase and serum α-L-Fucosidase in different treated groups. *= p= 0.001; **= p= 0.05.

Non-significant decreases in the levels of TAC, SOD, CAT and GSH were observed in liver and kidney tissues in MOE and MOE+AgNPs treated groups (fig. 4B, 4C, 4D and 4E respectively).

Liver and kidney functions

Treatment of animals with acetone (extract solvent) and solvent containing extract (MOE and MOE+AgNPs) resulted in a significant ($p<0.05$) changes in serum ALT and AST levels when compared to the control groups at numerical but these changes still within the normal range (fig. 4F). Levels of urea and creatinine in sera were in normal range as compared to untreated control (fig. 4G).

Serum tumor markers

The levels of serum α-L-Fucosidase and Arginase (fig. 4H) reflected non-significant increase in all treated groups.

DISCUSSION

Moringa plant is widely used and is called the miracle plant. In the current study, the main aim was to evaluate the biological properties of MOE, which is not tested before. Also, we compared the effects of that extract on colon cancer cells (HT-29 cells) for the first time with normal fast dividing cells (activated to proliferate).

In the current study, the biomolecules found in MOE were evaluated using FTIR. The test revealed the presence of many active biomolecules in the extract. Diverse phytochemicals exist in *M. oleifera* Lam. which are disseminated into all of its parts (root, leaves, seed, branches and pods). It was shown that several phytochemicals are present in *M. oleifera* including p-hydroxybenzyl glucosinolates (sinalbin), benzyl glucosinolates (glucotropaeolin), fatty acid, phenolics, 2-phnylethyl glucosinolates (gluconasturtiin), crude fats, major nutrient, flavonoids, total protein and mineral (Angasa *et al*., 2020). The seeds have high protein content while the leaves usually have high flavonoid value (Angasa *et al*., 2020). When comparing the fresh and dried leaf extracts, the extract obtained from dried leaves contained higher minerals, amino acid, and vitamin content and consequently a higher yield after purification (Angasa *et al*., 2020).

In the current study, AgNPs were synthesized using MOE. Other researchers could green synthesize AgNPs through aqueous *Moringa oleifera* leaf extract which assisted in particles formation and steadying through its reduction and stabilization power (Paul *et al*., 2020).

In the current study, neither proteins nor sugars were detected in MOE. We expected to find several protein bands in the acrylamide gel as leaves are reported to have considerable amount of protein (Jongrungruangchok *et al*., 2010). Also, other researchers estimated the percent carbohydrates in *Moringa oleifera* leaves and found it contain considerable amount of it.

In the traditional medicine, *M. oleifera* is usually used as ingredients to act as anti-bacteria and anti-tumor in pharmaceutical preparations (Anwar *et al*., 2007). Regarding the pharmaceutical industries, ingredients of this plant, in combination with other ingredients, are used to produce drugs (Al-Asmari *et al*., 2015).

In the time that many researcher (Ghosh *et al*., 2014) showed that phytochemicals of *M. oleifera* have biological properties including antimicrobial and anti-cancer, MOE and MOE+AgNPs, showed very weak antibacterial activity. Silver nanoparticles produced by extracts of the plant have significant applications in biology including antibacterial properties. Antimicrobial behaviors of AgNPs against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* has been evaluated (Rai *et al*., 2009). In the present study, the method of synthesis of AgNPs and its presence in the MOE may abrogated the AgNPs from acting on bacterial cells.

In the present study, ROS content in MOE was quantified. Results demonstrated a considerable amount of ROS in MOE. The target of estimating the ROS content in MOE was to know the extrinsic factors that may affect other parameters being investigated in this study like apoptosis.

Testing of plant extract cell cytotoxicity is very important and its evaluation needs controls for its evaluation. In the present study, we used activated splenocytes as comparable cell controls because most of the studies use normal control cells to test the anti-cancer activity against cancer cells. Normal cells are different from cancer cells at least in the rate of cell division. Cell cycle-related genes are generally mutated in tumors leading to the production of tumor growth (Williams and Stoeber, 2012). Cancer cells jump to G1 directly after M phase causing abnormal growth. Arresting the cell cycle at the M-phase is the target of most anti-cancer drugs. In the present study we simulated cancer cells by creating normal fast dividing cells (without gene errors) to be, for the first time, a good control for testing of anticancer behavior of any material.

MOE and MOE+AgNPs prepared in the present study exerted cell growth inhibitory effects on human colon cancer HT-29 cell line. A previous study reported that AgNPs contained in *M. oleifera* extract could induce apoptosis in cervical cancer cells through the increase in ROS production and other accompanied actions. Previously, it was reported that AgNPs could elevate the rate of ROS production through the inhibition of cell's replication (Vasanth *et al*., 2014). In the present study, extrinsic ROS was shown to be included in the extract. MOE and MOE+AgNPs possess no toxicity/cell cycle interference effects. This clearly indicates that MOE interferes cell cycle of cancer cells but not of normal fast dividing cells. Also, in the present study, MOE showed no cytotoxic effects on normal splenocytes indicating its safe effects on normal cells.

Studies reported that the bioactive compounds present in *M. oleifera* are capable of inducing apoptosis in tumor cells (Waterman *et al*., 2014). The cellular tumor suppressor protein, p53, plays a significant role in the apoptosis induction. It also control the pathway of the mitochondrial intrinsic apoptosis (Nieminen *et al*., 2013; Vaseva and Moll, 2009). When the cell losses or has inactivated p53, cancer diseases occur (Kundu *et al*., 2014). Apoptosis induction can be stimulated by biomolecules present in plants through the p53 activation (Luo *et al*., 2011). In the present study, p53 expression increased after treating the HT-29 cancer cells with MOE and MOE+AgNPs. Another study revealed that leaf extracts of *M. oleifera* could keep the viability of normal cell up to 70-90 % (Aboulthana *et al*., 2021; Angasa *et al*., 2020), indicating that *M. oleifera* displays a high power to control the p53 protein pathway when inducing apoptosis.

In the present study, MOE and MOE+AgNPs arrested HT-29 cancer cells at G2/M phases of the cell cycle. Previous studies demonstrated that dichloromethane and methyl alcohol leaf extracts of *M. oleifera* inhibited the cell division of hepatic (HepG2) and breast (MCF-7) cancers and human fibroblast cells (Gopalakrishnan *et al*., 2016; Suphachai, 2014).

In the present study, safety of MOE and MOE+AgNPs was tested *in vivo*. Kidney and liver markers reflected the safety of extracts. Some researchers (Adedapo *et al*., 2009) assessed the safety of the *M. oleifera* aqueous leaf extract (up to 2000 mg/kg) and showed that it is relatively safe. Others (Asiedu-Gyekye *et al*., 2014) also tested early-acute (40–1000 mg/kg) and acute (5000 mg/kg) toxicity in animals and demonstrated that no adverse effects was seen.

One of the cancer causative agents is the oxidative stress. It is an imbalance in the formation of oxidants and free radicals and their improper removal by antioxidants

(Reuter *et al*., 2010). Antioxidants have the power to interrupt the production of free radicals and downregulate the oxidative stress, leading to the prevention of cancer formation. In the current study, antioxidant activity of MOE and MOE+AgNPs was evaluated and results revealed that both of the extracts could modify the levels of antioxidant parameters studied in both liver and kidney. These results are in agreement with Alam *et al*. (2013) where they tested eight different extracts of *M. oleifera* leaves and showed that these extracts have antioxidant activities with different degrees. Also, many other researchers demonstrated that of *M. oleifera* leaves extract, prepared using several different solvents and methods, have antioxidant activities (Shih *et al*., 2011; Siddhuraju and Becker, 2003; Suphachai, 2014).

In the present study, cancer induction by MOE was tested *in vivo*. The levels of serum Arginase and serum α-L-Fucosidase after treatment with the extract and MOE+AgNPs and results showed that the levels of these tumor markers were lower than that of control untreated group.

CONCLUSION

Acetone preparations of *M. oleifera* leaves contained ROS and undetectable amounts of sugars and proteins and reflected a very weak antibacterial activity. MOE has the power to synthesize AgNPs with 61 nm diameter. For the first time, anticancer activity were tested on both abnormal (caner) and normal fast dividing cells. MOE and MOE+AgNP arrested cellular division of HT-29 cancer cells but not of normal fast dividing splenocytes indicating its capacity as specific anticancer agent. MOE and MOE+AgNP have antioxidant properties and induced apoptosis in HT-29 cancer cells rather than necrosis. The edible plant *M. oleifera* can be used to produce anticancer preparation at least against colon cancer as it is shown to be targeted and effective.

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REFERENCES

Abd Karim NA, Ibrahim MD, Kntayya SB, Rukayadi Y, Hamid HA and Razis AFA (2016). *Moringa oleifera* Lam: Targeting chemoprevention. *Asian Pacific J. Cancer Prev*., **17**(8): 3675-86.

Aboulthana WM, Shousha WG, Essawy EAR, Saleh MH and Salama AH (2021). Assessment of the anti-cancer efficiency of silver *Moringa oleifera* leaves nano-extract against Colon Cancer Induced Chemically in Rats. *Asian Pac. J. Cancer Prev*., **22**(10): 3267-3286.

Adedapo AA, Mogbojuri OM and Emikpe BO (2009). Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. *J. Med. Plants Res*. **3**(8): 586-591.

Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H and Islam M (2015). Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. *PLoS One,* **10**(8) e0135814.

Al Syaad KM and Ibrahim EH (2020). Study of the antioxidant, immunomodulatory and antibacterial properties of *Origanum majorana* leaf acetone extract. *Pak. J. Pharm. Sci*., **33**(5): 2209-2218.

Alam MN, Bristi NJ and Rafiquzzaman M (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm. J.,* **21**(2): 143-152.

Angasa TD, Hong Y, Ding Z and Ding X (2020). Anticancer effect of *Moringa oleifera* leaf extract on A2780 ovarian cancer cell line. *Asian J. Res. Med. Pharm. Sci.,* **9**(1): 1-6.

Anwar F, Latif S, Ashraf M and Gilani AH (2007). Moringa oleifera: A food plant with multiple medicinal uses. *Phyther. Res*., **21**(1): 17-25.

Asiedu-Gyekye IJ, Frimpong-Manso S, Awortwe C, Antwi DA and Nyarko AK (2014). Micro-and macroelemental composition and safety evaluation of the nutraceutical *Moringa oleifera* leaves. *J. Toxicol.* **2014**: 1-13.

Ganesan T, Sinniah A, Chik Z and Alshawsh MA (2020). Punicalagin regulates apoptosis-autophagy switch via modulation of annexin a1 in colorectal cancer. *Nutrients* **12**(8): 1-17.

Ghosh N, Paul S and Basak P (2014). Silver nanoparticles of *Moringa oleifera* - Green synthesis, characterisation and its antimicrobial efficacy. *J. Drug Deliv. Ther.,* **14**(1): 24-44.

Ghramh HA, Ibrahim EH and Kilany M (2020). Study of anticancer, antimicrobial, immunomodulatory, and silver nanoparticles production by Sidr honey from three different sources. *Food Sci. Nutr*. **8**(1): 445-455.

Gopalakrishnan L, Doriya K and Kumar DS (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Sci. Hum. Wellness* **5**(2): 49-56.

Ibrahim EH, Alshehri A, Ghramh HA, Kilany M, Morsy K, El-Kott AF and Sayed MA (2021). Rosemary extract and its biogenic silver nanoparticles induce apoptosis and arrest cell cycle in HT-29 colon cancer cells. *Sci. Adv. Mater*., **13**(1): 36-49.

Ibrahim EH, Kilany M, Ghramh HA, Khan KA and ul Islam S (2019). Cellular proliferation/cytotoxicity and antimicrobial potentials of green synthesized silver nanoparticles (AgNPs) using Juniperus procera. *Saudi J. Biol. Sci*., **26**(7): 1689-1694.

Jongrungruangchok S, Bunrathep S and Songsak T (2010). Nutrients and minerals content of eleven different samples of *Moringa oleifera* cultivated in Thailand. *J Heal. Res* **24**(3): 123-127.

Khor KZ, Joseph J, Shamsuddin F, Lim V, Moses EJ and Samad NA (2020). The cytotoxic effects of moringa oleifera leaf extract and silver nanoparticles on human Kasumi-1 cells. *Int. J. Nanomedicine,* **2020**(15): 5661-5670.

Kundu J, Chun KS, Aruoma OI and Kundu JK (2014). Mechanistic perspectives on cancer chemoprevention/ chemotherapeutic effects of thymoquinone. *Mutat. Res. - Fundam. Mol. Mech. Mutagen*. **768**(1): 22-34.

Luo H, Rankin GO, Li Z, DePriest L and Chen YC (2011). Kaempferol induces apoptosis in ovarian cancer cells through activating p53 in the intrinsic pathway. *Food Chem*. **128**(2): 513-519.

Madi N, Dany M, Abdoun S and Usta J (2016). *Moringa oleifera*'s nutritious aqueous leaf extract has anticancerous effects by compromising mitochondrial viability in an ROS-dependent manner. *J. Am. Coll. Nutr*. **35**(7): 604-613.

Matic I, Guidi A, Kenzo M, Mattei M and Galgani A (2018). Investigation of medicinal a dietary plants review traditionally supplements: On moringa used oleiferaas. *J. Public Health Africa* **9**(841): 191-199.

Nieminen AI, Eskelinen VM, Haikala HM, Tervonen TA, Yan Y, Partanen JI and Klefström J (2013). Myc-induced AMPK-phospho p53 pathway activates Bak to sensitize mitochondrial apoptosis. *Proc. Natl. Acad. Sci. USA* **110**(20): E1839-E1848.

Paul S, Basak P, Majumder R, Mukherjee A, Ghosh J, Patra S and Jana NK (2020). Biochemical estimation of *Moringa oleifera* leaf extract for synthesis of silver nanoparticle mediated drug delivery system. *J. Plant Biochem. Biotechnol*., **29**(1): 86-93.

Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S and Saravanan M (2013). Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi,* Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. **108**(2013): 255-259.

Rai M, Yadav A and Gade A (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol. Adv*. **27**(1): 76-83.

Reuter S, Gupta SC, Chaturvedi MM and Aggarwal BB (2010). Oxidative stress, inflammation, and cancer: How are they linked? *Free Radic. Biol. Med*., **49**(11): 1603-1616.

Shih MC, Chang CM, Kang SM and Tsai ML (2011). Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the chemical compositions and antioxidant activity of *Moringa oleifera. Int. J. Mol. Sci*., **12**(9): 6077-6088.

Siddhuraju P and Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem*., **51**(9): 2144-2155.

Silveira C, Shimabuku QL, Fernandes Silva M and Bergamasco R (2018). Iron-oxide nanoparticles by the green synthesis method using *Moringa oleifera* leaf extract for fluoride removal. *Environ. Technol*. (United Kingdom) **39**(22): 2926-2936.

Suphachai C (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *J. Med. Plants Res.,* **8**(7): 318-325.

Tiloke C, Phulukdaree A, Chuturgoon AA (2016). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on human esophageal cancer Cells. *J. Med. Food,* **19**(4): 398-403.

Vasanth K, Ilango K, MohanKumar R, Agrawal A and Dubey GP (2014). Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids Surfaces B Biointerfaces,* **117**: 354-359.

Vaseva AV and Moll UM (2009). The mitochondrial p53 pathway. *Biochim. Biophys. Acta - Bioenerg*. **1787**(5): 414-420.

Vergara-Jimenez M, Almatrafi MM and Fernandez ML (2017). Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants.* **6**(4): 1-13.

Waterman C, Cheng DM, Rojas-Silva P, Poulev A, Dreifus J, Lila MA and Raskin I (2014). Stable, water extractable isothiocyanates from *Moringa oleifera* leaves attenuate inflammation *in vitro*. *Phytochemistry* **103**: 114-122.

Williams GH and Stoeber K (2012). The cell cycle and cancer. *J. Pathol*., **226**(2): 352-364.

Wright RJ, Lee KS, Hyacinth HI, Hibbert JM, Reid ME, Wheatley AO and Asemota HN (2017). An investigation of the antioxidant capacity in extracts from *Moringa oleifera* plants grown in Jamaica. *Plants* **6**(4): 1-8.

Wu YY, Xu YM, Y Lau AT, Ferreira de Oliveira P, Ribeiro D, Ascenso A and Santos C (2021). Anti-cancer and medicinal potentials of Moringa Isothiocyanate. *Mol*. **26**(24): 1-14.