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A POSSIBLE ROLE OF *MORINGA OLEIFERA* LEAVES EXTRACT ON MYELOID DERIVED SUPPRESSOR CELLS IN BENZENE-INDUCED LEUKEMIA

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ABSTRACT
Myeloid derived suppressor cells (MDSCs) are a diverse population of hematopoietic precursors with the ability to affect host immunity. They have been revealed to be upregulated in cancer. Limited knowledge is available on myeloid derived suppressor cells of rat origin. The exact role of MDSCs in benzene-induced leukemia (BIL) has not yet been effectively studied. Alternatively, medicinal plants are important medical systems that have persevered in developing countries. *Moringa Oleifera* (MO), one of the best known small native tree, has been studied as a potential therapeutic agent that has anti-cancer activity. Doxorubicin hydrochloride (Dox) has proved to have potential therapeutic responses in acute myeloid leukemia. The objective of this study is to evaluate the therapeutic action of *Moringa Oleifera* leaves extract versus Dox on the MDSCs in benzene-induced leukemia. To that goal 112 young adult male albino rats were allotted into two major groups, control and experimental where each was further divided into 4 sub-groups. Total leucocytic count, flow cytometry for $CD11bc^{+}/His48^{+}$ MDSCs were assessed in peripheral blood. Also, histological sections in blood, spleen, and liver were studied in all groups. Results of the current study confirmed that *Moringa Oleifera* leaves extract seems to be most promising in overcoming doxorubicin side effects and decreasing MDSCs in benzene-induced leukemia, thus providing a novel insight into the application of *Moringa Oleifera*-based treatments.

KEYWORDS: *Moringa Oleifera*, Myeloid Derived Suppressor Cells, Benzene-Induced Leukemia, Spleen.

INTRODUCTION
The incidence of deadly diseases is disturbing, which has geared a number of scientists into research on various causes of these diseases. One of the deadliest diseases currently killing humans and even animals is cancer. Cancer is a broad group of various diseases involving unregulated abnormal cell growth, develops in almost any organ or tissue and occurs in many forms of over 200 different types.[1] Leukemia, one of the most common forms of cancer in infants and adults, is a malignant clonal disorder characterized by alterations and low production of healthy hematopoietic cells. This alteration inhibits differentiation of cells and induces proliferation and accumulation of immature ones (blasts).[2] The accumulation of blasts begins in bone marrow, but in most cases quickly builds up in the blood, and sometimes spreads to other parts of the body such as the lymph nodes, spleen, liver, testes, and the central nervous system.[3] Generally, in cancer, the bone marrow microenvironment becomes immunosuppressive and plays a crucial role in development and progression of the disease. Immune suppression is caused by a limitation in proinflammatory cytokine production, inhibition of activated immune cells, and generation or expansion of immunosuppressive cell types such as myeloid-derived suppressor cells.[4]

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that regulate immune responses in healthy individuals and in the context of various diseases.[5] However, limited knowledge is available on myeloid derived suppressor cells of rat origin. Ordinarily, immature myeloid cells formed in the bone marrow differentiate to dendritic cells, macrophages and neutrophils. However, under chronic inflammatory circumstances or cancer, myeloid cells differentiation is skewed on the way to the expansion of MDSCs. These MDSCs can infiltrate inflammatory sites and tumors, where they stop immune responses by inhibiting T lymphocytes and NK cells. Therefore, they may become a key therapeutic target.[6] Inflammatory environments have been shown to induce MDSCs development and accumulation. Indeed, IL-6, VEGF (vascular endothelial growth factor) and MCF (macrophage colony factor) all promote the development of MDSC populations by activating the STAT3 (signal transducers and activators of transcription family3) pathway. IL-4 and GM-CSF, on the other hand, can inhibit MDSCs function by shifting them into mature

dendritic cells.[7] In human or murine models of disease, MDSCs have been shown to have roles in cancer, bacterial and parasitic infections, acute and chronic inflammation[7] and can support tumor growth through alteration in the tumor microenvironment.[5]

Several types of treatment may be used for people with leukemia. Doxorubicin Hydrochloride has produced significant therapeutic responses in carcinoma of the breast, lung and ovary, acute lymphocytic leukemia and acute myeloid leukemia.[8] The most dangerous side effects of doxorubicin are cardiomyopathy leading to congestive heart failure, typhlitis (an acute life-threatening infection of the bowel), fever, cough and congestion, or other signs of infection such as unusual bleeding; bloody stools and bloody vomiting.[9]

Because of these drugs adverse side effects, researches are focusing on phytomedicines that seem to have anti-cancer and immune system enhancing activity. Phytomedicines are believed to have benefits over conventional drugs and are regaining interest in current research. Some important anticancer agents are still extracted from plants as they cannot be manufactured chemically due to their complex structures.[10]

Moringa Oleifera (MO) is one of the best known and most widely distributed small native tree. It is now indigenous to many regions in North West India, Africa, Arabia, South East Asia, the Pacific and Caribbean Islands and South America. By tradition, besides being a daily used vegetable between people of these regions, it is also widely known and used for its health benefits.[10] Several phytochemical compounds have been isolated from MO such as quercetin, flavonoids, kaempferol, anthocyanins, carotenoids, vitamins, minerals, amino acids, sterols, glycosides, and alkaloids. Also, it contains a unique group of compounds called glucosinolates and isothiocyanates which have long been known for their bactericidal, nematocidal, fungicidal, allelopathic properties and cancer chemo-protectivity.[11]

Hamza,[12] discovered that the *Moringa Oleifera* seed extract exhibited antifibrotic effects on liver fibrosis in rats. Also, it was found to have blood pressure lowering effect which makes it useful in a cardiovascular disorder. In addition, Francis at al.,[13] demonstrated that *Moringa Oleifera* constitutes can be used for the treatment of diabetes mellitus by triggering insulin release from the rodent pancreatic cell. In addition, *Moringa* fruit has been found to lower cholesterol to phospholipid ratio, atherogenic index lipid and reduced the lipid profile of liver, heart, and aorta in hypercholesteremic rabbits.[14] It has been described to demonstrate antioxidant activity due to its high amount of polyphenols.[15] Additionally, Lipipun et al.,[16] illustrated that *Moringa Oleifera* extracts have antibacterial activity and are rich in antimicrobial agents. Interestingly, Bharali et al.,[17] presented evidence on the function of niazimicin (one of the *Moringa Oleifera* components), as a potent anti-tumor promoter. More to the point, Al-Asmari et al.,[10] demonstrated that *Moringa Oleifera* extracts act as an anti-cancer agent by decreasing cell mortality and colony formation in colorectal and breast cancer cell lines. In addition, seed extracts of *Moringa Oleifera* have been demonstrated to prevent skin tumors in mice.[17] Moreover, it was established that *Moringa Oleifera* roots were active against leukemia cells in vitro and could be used as natural antitumor medicines.[18] Additionally, MO leaves extracts showed great cytotoxic effects for tumor cells, strongly suggesting that it could be an ideal anticancer therapeutic candidate specific to cancer cells.[19]

The present work aims to evaluate the potential role of Myeloid-derived suppressor cells in progression of benzene-induced leukemia and the possible role of *Moringa Oleifera* in enhancing the therapeutic action and minimizing the adverse side effects of Doxorubicin Hydrochloride.

MATERIAL AND METHODS

Animals

112 young adult male albino rats were used in the present investigation. They were purchased from Theodor Bilharz Research Institute, Cairo, Egypt. All animal procedures were performed in accordance with the declaration of Helsinki and the guidelines for the care and use of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH) protocol. Their weights ranged between 150-180 gm representing 6-8weeks of age. Animals were allowed a one-week pre-experimentation period to adapt to laboratory conditions. They were housed in metabolic cages.

All animals were in perfectly good condition and health in order to avoid any other intruding factors upon the experiment. They received food and water *ad libitum* with fresh supplies presented daily.

Treatments and Dosage

a. Leukemia Induction

Leukemia was successfully induced in rats by intravenous injection of 0.2 mL of a 1:10 diluted benzene solution (Chromasolv, in water/2-propanol [50/50] v/v), given every 2 days for 3 consecutive weeks.[20]

b. Doxorubicin Hydrochloride

Doxorubicin hydrochloride (Sigma Chemical Co., St. Louis, MO) was administered by intraperitoneal injection at a dose of 10 mg / kg b.w. every other day for four weeks[21], while control animals were injected with a comparable volume of saline.

c. *Moringa Oleifera*

Fresh leaves of *Moringa Oleifera* plant were picked from trees grown on sand soil in El- Sharkia governorate, Egypt. The leaves were washed carefully with distilled

water and dried under room temperature for three weeks, after which they were minced into coarse form. The coarse form was then macerated in absolute ethanol. This was left to stand for 48 h. After that, the extract was filtered and the resulting ethanol extract was concentrated and evaporated to dryness at optimum temperature between 40 and 45°C to avoid denaturation of the active ingredients. The concentrated extract was diluted using polysaccharide as a carrier and stored in the refrigerator.[22] *Moringa Oleifera* ethanol extract was given daily by oral gavage at a dose of 680 mg/day for four weeks.[22]

Animal Grouping

The two major groups compromised a control and experimental one. The latter was injected with benzene for leukemia induction. Following 3 weeks post induction period each was further subdivided into 4 subgroups according to the following where dissections were performed after 2 and 4week interval after ether inhalation anaesthesia:-

1- Controls groups (14 animals each)
a) Normal control (C) given normal saline intraperitoneally.
b) Doxorubicin Hydrochloride group (DOX.) given intraperitoneally at a dose of 10 mg / kg b.w. every other day for four weeks.[21]
c) *Moringa Oleifera* group (MO) orally given MO extract at a dose of 680 mg/day for four weeks.[22]
d) Doxorubicin Hydrochloride and *Moringa Oleifera* group (DOX+MO).
2- Experimental groups (14 animals each) benzene-induced leukemia group (BIL)
a) Benzene-induced leukemia group (BIL)
b) (BIL/DOX.).
c) (BIL/MO).
d) (BIL /DOX+MO).

Individual body weights were monitored weekly where whole body weights were recorded to the nearest 1mg to determine body weight gain.

Determination of Total Leucocytic Count

After 2 and 4 weeks animals were sacrificed by ether inhalation anaesthesia. Blood samples were withdrawn by heart acupuncture from all groups in dry clean sample tubes containing EDTA for the determination of complete blood picture.

Histological Studies

Dissected organ specimens of liver and spleen were placed separately in 10% buffered formalin after washing with 0.9% saline solution. This was subsequently followed by processing, sectioning at 6 microns thick and routine staining with HX and E. for general histological analysis. Additionally, blood smear was performed on clean slides and stained by HX and E for histological studies.

Flow Cytometric Assessment of $CD11bc^{+}/His48^{+}$ MDSCs.

For flowcytometric analysis peripheral blood mononuclear cells were isolated and stained with a combination of Anti-Rat CD11bc PE (catalog number:12-0110, clone OX42) and Anti-Rat Granulocyte Marker Biotin FITC (catalog number: 13-0570, clone His48). Then the tubes were mixed well using vortex and incubated for 20 minutes in the dark at room temperature. After lysing red cells, cells were analyzed by flow cytometer (Coulter EPICS-XL flow cytometer system for 3 color fluorescence detection with catalog number 4327296). A coulter flow center multimedia work station was used as an offline computer system for data analysis.

Statistical Analysis

Statistical analysis was done using the statistical package for social science (SPSS) version 19 statistical software (ANOVA) followed by Tukey's post-test. Data are expressed as the mean ± standard error of mean (SEM). *P < 0.05 for significant results compared to control group. #P < 0.05 for significant results treatment compared to benzene-induced leukemia group.

RESULTS

Average growth rates

Body weight gain in control animals (C) and (MO) evidenced more or less gradual significant increase reaching 212.2 gm and 247.7 gm respectively at the end of the study. Nevertheless, DOX group showed remarkable decrease in weight recording 127 gm that was more ameliorated when MO was given with DOX recording 188.3 gm. On the other hand, as BIL rats suffered severe decrease in body weight gain reaching 136.3 gm, yet given DOX induced still more decrease recording 124.7 gm (Table 1). Nevertheless, as MO was introduced to rats it managed to induce significant increase in body weight gain reaching 25.5% and 11.2% in BIL/MO and BIL/DOX+MO as compared to their initial weights highlighting the important role of *Moringa Oleifera* in improvement of body weight (Table 1).

Table (1): Averages of growth rates in control and experimental groups.

Week NO.	Group	Control	DOX	MO	DOX+MO	BIL	BIL /DOX	BIL /MO	BIL /DOX+MO
W1	Mean±S.E	177±2.8	172.7±2.3	177.9±2.2	171.0±1.7	154.4±1.6	156.2±2.4	156±3.06	159.8±4.6
	%of change		-2.43	0.51	-3.39	-12.77	1.17	1.04	3.50
	P Value		N.S.	N.S.	N.S.	P<0.05	N.S.	N.S.	N.S.
W2	Mean±S.E	192.8±2.7	165.7±3.9	196.2±2.5	172.8±2.2	156.2±7.5	149.8±1.6	163.8±1.9	166.3±2.3
	%of change		-14.06	1.76	-10.37	-18.98	-4.10	4.87	6.47
	P Value		N.S.	N.S.	N.S.	P<0.05	N.S.	N.S.	P<0.05
W3	Mean±S.E	207.1±10.8	141.8±4.4	224.3±2.01	185.5±5.7	146.8±4.7	134.7±4.9	177.7±10.2	171.8±4.6
	%of change		-31.53	8.31	-10.43	-29.12	-8.24	21.05	17.03
	P Value		P<0.05	N.S.	P<0.05	P<0.05	N.S.	P<0.05	P<0.05
W4	Mean±S.E	212.2±6.6	127±6.5	247.7±1.5	188.3±4.9	136.3±4.01	124.7±5.4	195.8±3.4	177.7±6.3
	%of change		-40.15	16.73	-11.26	-35.77	-8.51	43.65	30.37
	P Value		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Total Leucocytic Count

Ten animals from both normal control and BIL group were examined for leukemia induction after 3 weeks of benzene injection by CBC test. A high WBC count of 39.5 was considered a positive sign of induction in comparison to normal WBC count of 8.85. After 2 weeks from the beginning of the treatment (Table 2), BIL group showed a significant increase in WBC count when compared to control group which was an indication of successfully-induced leukemia. In all treated groups, WBC count significantly decreased when compared to BIL group. Notably, BIL\DOX+MO showed the most ameliorated results. Similar amended results occurred after 4 weeks (Table 2).

Table (2): White blood cell count in control and experimental groups.

Parameter	Group							
WBC Count ($X10^3$)	Control	DOX	MO	DOX+MO	BIL	BIL /DOX	BIL /MO	BIL /DOX+MO
Zero Time	8.87±0.09	-------	------	------	39.5±0.18*	------	------	------
2 weeks	9.28±0.27	11.00±0.26	6.25±1.56	10.11±0.95	30.35±5.53*	17.11±2.06#	12.35±3.70#	8.93±3.85#
4 weeks	9.73±0.50	11.62±0.25	7.22±1.41	8.75±1.23	22.37±2.16*	11.57±3.74#	8.90±3.28#	7.20±2.36#

*P < 0.05 for significant results compared to control group.

#P < 0.05 for significant results treatment compared to benzene-induced leukemia group.

Histological Observations

a- **Blood Smears**

In BIL group, the most prominent feature characterizing blood smears was the appearance of excessive white blood cells compared with the control groups (leukocytosis) (Fig. 1b). This gradually attenuated with the lapse of time as animals were given the different curative measures being mostly evident in the BIL/DOX+MO rats that showed near to normal distribution of WBCs.

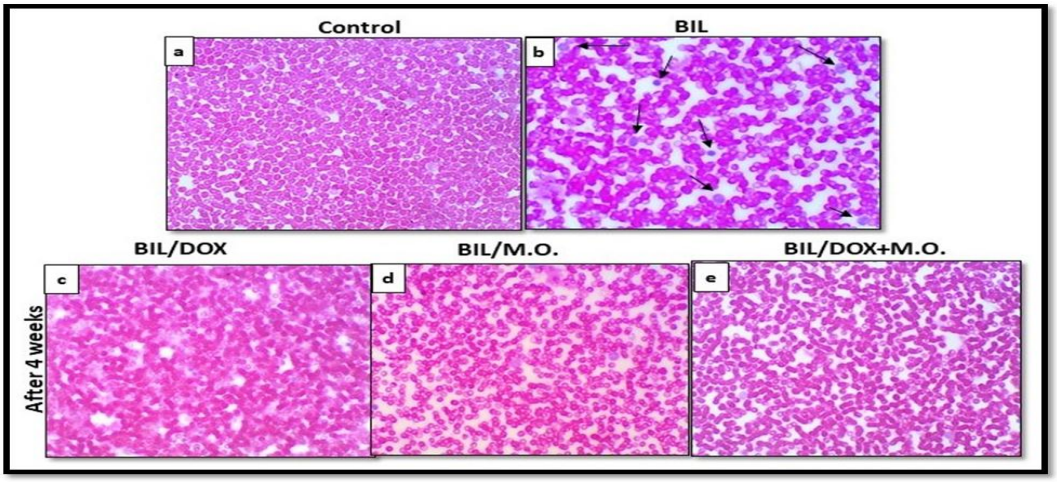

Figure (1): Photomicrograph of blood smears of rats in different experimental groups stained by H&E. White blood cells (arrow). Original magnification, 10X.

b- Liver

Hepatic sections from normal control rats exhibited normal architecture liver throughout the experimental duration (Fig. 2a). Nevertheless, rats administered DOX identified degenerative changes in both nuclei and cytoplasm, sinusoidal spaces were dilated and invaded by clumps of eosinophilic inflammatory cells with prominence of inflammatory Kuppfer cells (Fig. 2b). In BIL group, sinusoidal spaces were dilated with appearance of inclusions in hepatocytes and necrotic nuclei (Fig. 2c). Following treatment with DOX, liver sections taken from rats after 2 weeks designated necrotic hepatic cells. Some nuclei appeared pyknotic with clumped nuclear material that progressed to karryolysis in others (Fig. 2d). Other areas suffered from disintegration in cellular membrane where cells appeared fused together. Such alterations progressed to loss of hepatic pattern after 4 weeks of DOX to BIL rats (Fig. 2g). As MO was introduced to experimental rats altered hepatic lesions were still detected in liver tissue. These changes included degenerative hepatocytes and dilated sinusoidal spaces with prominent augmented presence of Kuppfer cells (Fig. 2e). 4-week treatment duration was not able to diverge induced lesions completely but limited ameliorated figures were encountered (Fig. 2h). Again, under the double stress of BIL and DOX, MO leaved failed to stimulate curative measures where amorphous material invaded some cells that showed karryorrhexis in nuclei (Fig. 2f). Inspite of limited amended figures after 4 weeks yet pyknotic nuclei persisted with dilated sinusoidal spaces (Fig. 2i).

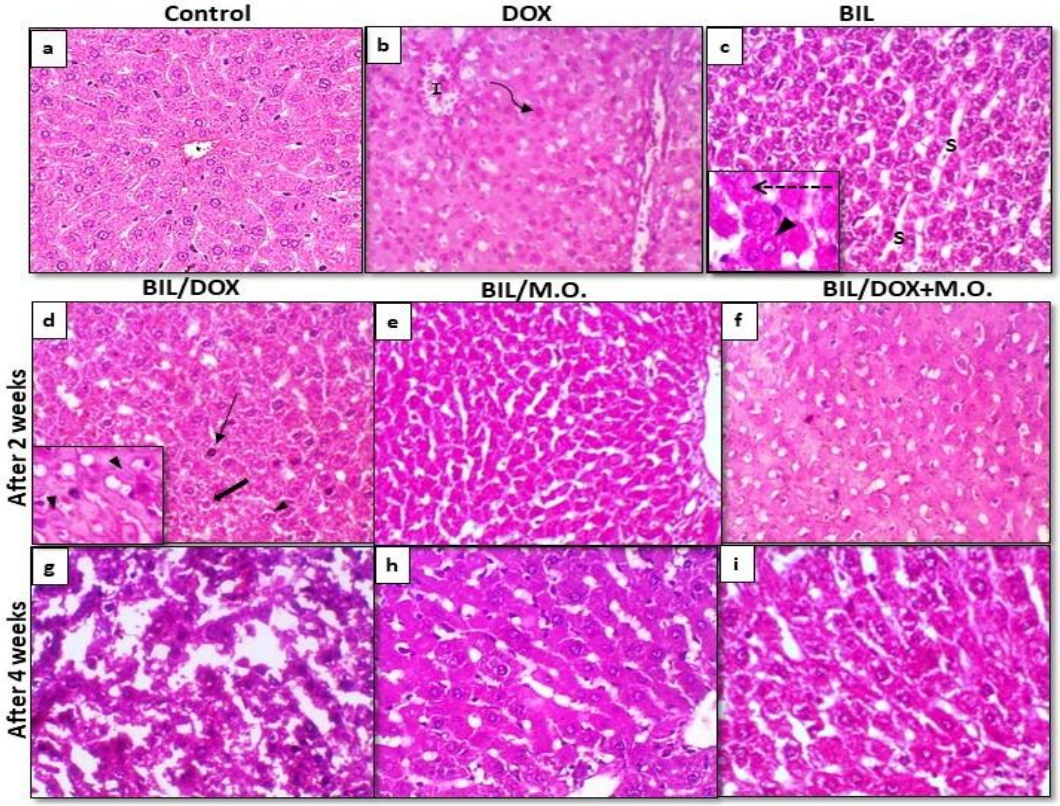

Figure (2): Photomicrograph of a section in the liver of rat stained by H&E. Inflammatory cells infiltration (I), degenerative changes in hepatocytes (curved arrow); sinusoidal dilatation (S); necrotic nuclei (arrow head); inclusions in hepatocytes (dashed arrow); pyknosis (thin arrow); karyolysis (thick arrow). Original magnification, 10X; 40X in high power.

c- Spleen

Splenic tissue from control animals manifested normal patterns throughout the experimental duration (Fig. 3a). Nevertheless, introduction of DOX to animals managed to induce limited haemorrhagic changes intersecting splenic trabeculae in the white pulp areas and increase in fibrous tissue (Fig. 3b) which was ameliorated after MO administration in DOX+MO group (Fig. 3c). In BIL group, sinusoidal spaces were dilated with appearance of hemorrhagic lesions and distortion of splenic architecture (Fig. 3d).

After treatment, histopathological alterations in spleen sections taken from BIL/DOX group showed persistent hemorrhagic lesions and dilatation in sinusoids (Fig. 3e) which increased after four weeks showing partial distortion of splenic architecture with increase lymphocytic inflammatory cells (I) (Fig. 3h).

In BIL/MO group, minimal hemorrhage and degeneration in red and white pulp was observed except for minimal aggregations of deeply stained lymphocytes in red pulp (I) (Fig. 3f) which was partially ameliorated after four weeks of MO administration (Fig. 3i). In BIL/DOX+MO group, there was a great improvement in splenic architecture either after two weeks (Fig. 3g) or after four weeks (Fig. 3j) of treatment. While Malpighian lymphoid corpuscles were more or less atrophic after 2 weeks, yet such lesion soon resided after 4 weeks.

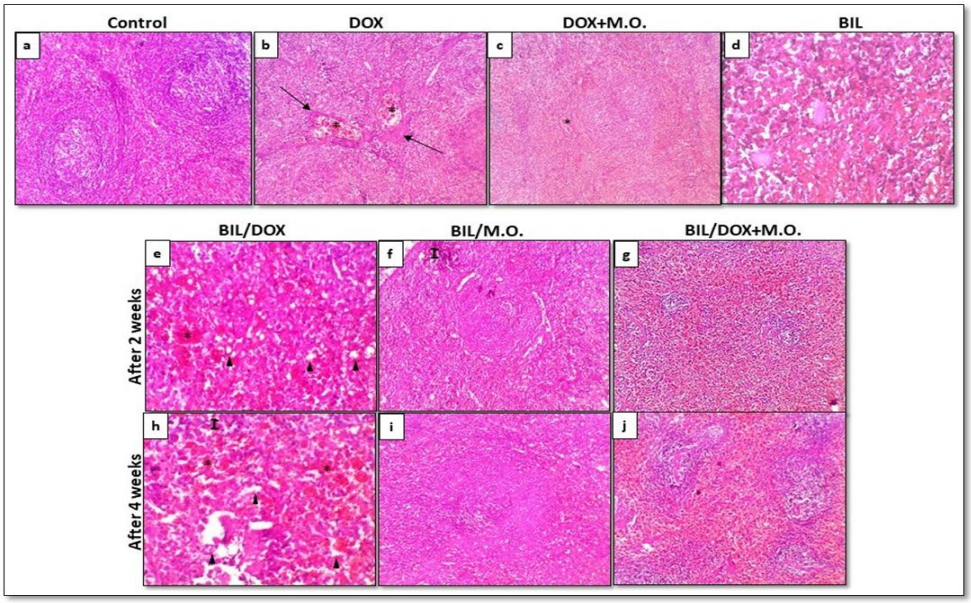

Figure (3): Photomicrograph of a section in the spleen of rat stained by H&E (10 X). Hemorrhage (*), Fibrosis (arrow), lymphocytic inflammatory cells (I) and dilatation in sinusoids (arrow head), Inflammatory cells infiltration (I). Original magnification, 10X.

Flow Cytometric Assessment of CD11bc^{+}/His48^{+} MDSCs.

Flowcytometry studies using CD11bc and His48 antibodies were performed on blood cells from control and benzene-induced leukemia rats at the end of the experiment. As seen in figure 4, the percentage of CD11bc/His48 cells in BIL group is significantly increased compared to control group. After treatment, the percentage of CD11bc/His48 cells was significantly decreased related to BIL group. Notably, BIL/DOX+MO group showed the best ameliorative results.

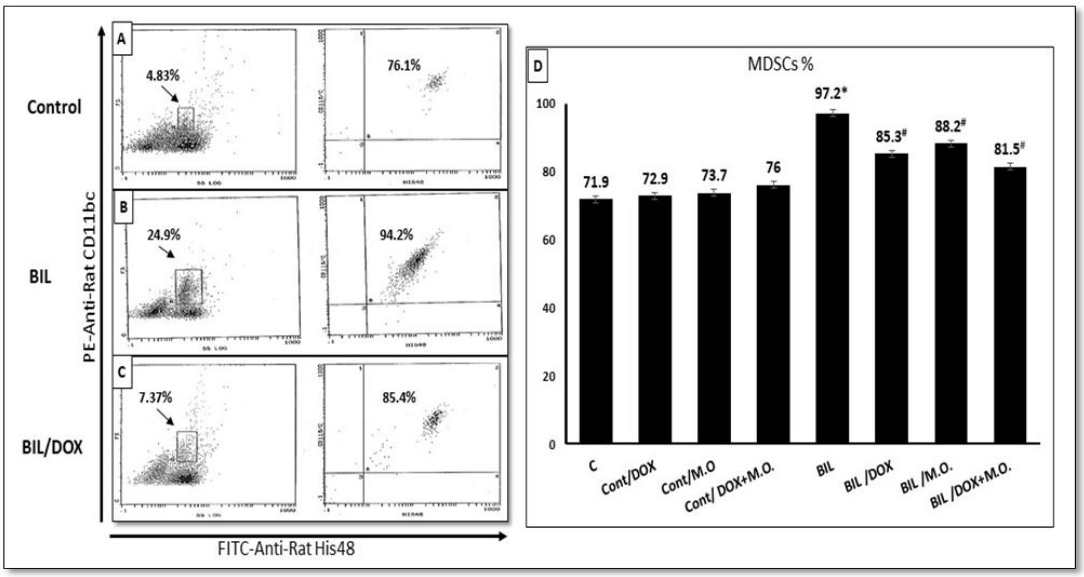

Figure (4): Flow cytometry analyses of CD11bc+/His48+ cells. (A) Representative flow cytograms of one animal from the control group. (B) Representative flow cytograms of one animal from BIL group. (C) Representative flow cytograms of one animal from BIL/DOX treated group. The percentage of CD11bc+/His48+ cells in each sample is shown in the upper right quadrant of each flow cytogram. (D) Percentages of CD11bc+/His48+ MDSCs cells in different experimental groups. Data are means ± standard error. *P < 0.05 for significant results

compared to control group. #P < 0.05 for significant results treatment compared to benzene-induced leukemia group.

DISCUSSION

In the present study, normal control animals showed a gradual increase of 19.9% in body weight throughout the experiment duration with a decrease of 11.7% in BIL animals.[23] This significant decrease in body weight (from 154.4±5.4 to 136.3 gm, P<0.05) in BIL group confirmed the development of leukemia in rats (Table 1). Administration of doxorubicin in either DOX group or in BIL/DOX group led to a severe reduction of body weight that recorded 26.5% and 20.2% respectively compared to their initial weights. Similar findings were reported by several authors.[24] This decrease in body weight may be attributed to the decline in food consumption.[25] In addition, decrease in body weight may be due to increased inhibition of DNA synthesis pathway, hence, doxorubicin is a cell cycle-nonspecific agent that acts by blocking topoisomerase II activity and by intercalating into the flat space between the bases of the DNA double helix, where it can act further to disrupt DNA replication and transcription.[26]

On the other hand, when doxorubicin was combined with *Moringa Oleifera*; enhanced results were manifested recording increase of 10.1% and 11.2% in DOX+MO and BIL/DOX+MO groups respectively compared to their initial weights. Moreover, *Moringa Oleifera* administration alone in MO and BIL/MO groups showed a continuous increase in body weights with the lapse of time that amounted to 39.2% and 25.5% respectively compared to their initial weights. These results are in agreement with Osman et al.,[27] and Alabi et al.,[28] who provided evidence that the positive effect of *Moringa Oleifera* in body weight may be due to the high crude protein content in it. Also, this increment in body weight might be as a result of the pharmacological chemical compounds (carbohydrates, saponins, cardiac glycosides, terpenes, steroids, flavonoids, and alkaloids) present in the *Moringa Oleifera* extract reported by.[29] Furthermore, leaves of *Moringa Oleifera* are rich in carotenoids, vitamins, amino acids, alkaloids, flavonoids and minerals particularly iron.[30,31] Moreover, the significant increase in body weights of rats might also be attributed to captivity, where energy spending is minimal.[32] In addition, Khan et al.,[33] proved that supplementation of *Moringa Oleifera* leaf powder in diet promoted gut health through improved intestinal microarchitecture and cellular count.

Leukocytosis was observed in the groups of benzene-induced leukemia rats as demonstrated in some other works[20] (Fig. 1 and Table 2). Leukocytosis is usually associated with leukemia and was found to be restored or prevented in treated rats (Table 2). There was a significant decrease in WBC count in BIL/DOX group when compare to BIL group either after two weeks or four weeks from treatment. However, doxorubicin is wildly used as a chemotherapeutic agent due to its ability to induce apoptosis. The apoptosis pathway is triggered when the attempt to repair the breaks in nuclear DNA fail and cellular growth is inhibited at phases G1 and G2.[34] Also, it was reported doxorubicin's ability to intercalate with not only nuclear DNA but also mitochondrial DNA.[35] Other doxorubicin actions include free radical generation which causes further DNA damage, inhibition of macromolecule production, DNA separation and increase in alkylation.[36] Furthermore, doxorubicin can affect the cell membrane directly by binding to plasma proteins causing enzymatic electron reduction of doxorubicin. This can cause the formation of highly reactive species of hydroxyl free radicals.[34] Additionally, it was explored that doxorubicin can decline antiapoptotic Bcl-2 with elevation in proapoptotic Bax in vitro studies on breast cancer cell line MCF-7.[37] However, the main complication of chemotherapy is that it cannot maintain normal cells without damaging, due to that its blinded compounds have no ability to differentiate between tumor and normal cells. Doxorubicin induces apoptosis and necrosis in healthy tissue causing toxicity in the brain, liver, kidney, and heart. This often impacts the efficacy of the therapy, making it impossible to cure patients suffering from cancer.[38]

In the present study liver and spleen histological examinations demonstrate this toxic effect of doxorubicin. The present examination of the liver of DOX and BIL/DOX rats revealed degenerative changes in hepatocytes; necrosis; pyknosis and karyolysis in hepatic parenchymal cells which rises after four weeks displaying loss of hepatic architecture (Fig. 2). Also, the present examination of the spleen of DOX and BIL/DOX rats revealed degenerative changes in spleen cells; internal hemorrhage in red pulp with an increase in fibrous tissues; hemorrhage and dilatation in sinusoids which increases after four weeks showing partial distortion of splenic architecture with necrotic areas (Fig. 3). These toxic effects are attributed to that doxorubicin-mediated redox cycling and therefore ROS and free radical generation are responsible for its toxicity.[39]

Because of these drugs' adverse side effects, researchers are focusing on phytomedicines that seem to have anti-cancer and immune system enhancing activity. Phytomedicines are believed to have benefits over conventional drugs and are regaining interest in current research.[10] *Moringa Oleifera* is considered to be a main medicinal plant.[30]

Regarding liver tissue histological observations, in BIL/DOX+MO group, there was a limited improvement in hepatic architecture after four weeks as compared to treatment with doxorubicin alone in BIL/DOX group (Fig. 2). Spleen sections enhancement was designated in groups treated with MO. This was marked in the form of minimal hemorrhage and degeneration in red and white pulp cells which was ameliorated after four weeks of MO

administration. In BIL/DOX+MO group, there was a great improvement in splenic architecture either after two or four weeks of treatment. These improvements are due to neutralization of the ROS and free radicals which are generated from doxorubicin therapy. Free radical scavenging represents one of the important characteristics of *Moringa Oleifera* leaves extract which is rich in antioxidants. Antioxidants provide protection or remediation by scavenging reactive oxidative species (ROS) that initiate diseases such as cancer.[40] Also, many components of *Moringa Oleifera* leaves extract such polyphenols and various carotenoids were observed to improve the immune system, scavenge free radical and reduce the production of DNA mutations that were previously exposed to a variety of oxidative conditions.[41]

More to the point, there was a significant decrease in WBC count in BIL/MO group when compared to BIL group either after two weeks or four weeks from treatment in the present study. *Moringa Oleifera* has potent antiproliferative activity and apoptosis inducing capacity on tumor cell line. It also increases the cytotoxicity of chemotherapy on pancreatic cancer cells.[42,43] The mechanisms of anticancer properties of *Moringa Oleifera* is still emerging. Akanni et al.,[44] concluded that enhanced apoptosis by increased expression of TNF-α is a possible mechanism of action of *Moringa Oleifera* extract in rats bearing benzene-induced leukemia. TNF-α activates and increases NK cytolytic function and can induce apoptosis through the activation of Type I receptors, which are considered part of an apoptotic cascade. Recently, *Moringa Oleifera* leaves and roots extracts act as anti-cancer agent by decreasing cell proliferation and exhibiting apoptosis-mediated cell death in liver HepG2, colon HCT 116 and Caco-2 and breast MCF7 cancer cell lines.[18] Furthermore, polyphenols; present in MO leaves extract; were shown to inhibit a specific protein responsible for cancer in bone and also increase the production of antioxidants in the sperms.[45] Moreover, it was proved that co-treatment with kaempferol and quercetin; compounds found in *Moringa Oleifera;* showed inhibition of cell proliferation of human HuTu-80 duodenum adenocarcinoma cells, human Caco-2 colon cancer cells and human PMC42 breast cancer cells.[46] Tragulpakseerojn te al.,[47] proved that kaempferol was reported to induce apoptosis in HCT116 colon cancer cells through induction of caspase-3 which is considered as a crucial component of the apoptotic machinery.

However, the mechanisms of anticancer properties of *Moringa Oleifera* is still developing. The current study supposed a new anticancer mechanism of *Moringa Oleifera* by decreasing myeloid derived suppressor cells (MDSCs). In the present work, after treatment with MO, the percentage of MDSCs was significantly decreased compared to BIL group (Fig. 4) that may be attributed to the apoptotic action of MO.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that compose a unique constituent of the immune system that regulate immune responses in healthy individuals and suppress T cell immunity in tumor-bearing hosts. They are expanded in various disease states including cancer.[5]

In the present study, flowcytometric analyses for MDSCs were performed at the end of the experiment. The percentage of MDSCs in BIL group was significantly increased compared to control group (Fig. 4). These results were in accordance with Dolen et al.,[48] who verified that MDSCs numbers were expanded in peripheral blood and spleens of mammary tumor-bearing rats. Moreover, an increased population of MDSCs in the peripheral blood of hepatocellular carcinoma patients was observed.[49] Additionally, it was identified that there is a significantly expanded MDSCs population in chronic myeloid leukemia patients.[50] Also, Zhang et al.,[51] substantiated the presence of increased immunosuppressive circulating and tumor-resident MDSCs in patients with colorectal cancers correlating with cancer stage and metastasis. Similarly, Van Valckenborgh et al.,[52] have demonstrated that immunosuppressive MDSC subsets were present and active in mice model of Multiple myeloma.

MDSCs are induced by tumor-secreted and host-secreted factors, this accumulation of MDSCs down-regulate immune surveillance and antitumor immunity, thereby facilitating tumor growth.[53] MDSCs express high levels of both arginase and iNOS. The increased activity of arginase leads to enhanced L-arginine catabolism, which lead to inhibition of T-cell proliferation.[54] Another important factor that contributes to the suppressive activity of MDSCs is ROS. Several known tumor-derived factors, such as TGFβ, IL-10, IL-6, IL-3, platelet-derived growth factor and GM-CSF, can induce the production of ROS by MDSCs.[55] In addition, it has emerged that peroxynitrite (ONOO-) is a crucial mediator of MDSC-mediated suppression of T-cell function and one of the most powerful oxidants produced in the body. It was demonstrated that peroxynitrite production by MDSCs during direct contact with T cells results in nitration of the T-cell receptor (TCR) and CD8 molecules, which alters the specific peptide binding of the T cells and renders them unresponsive to antigen-specific stimulation.[56] More to the point, the ability of MDSCs to promote the de novo development of regulatory T cells in vivo has been described.[57] Further mechanisms of T cell inhibition mediated by MDSCs include the findings from the Ku et al.,[58] who demonstrated that MDSCs facilitate the downregulation of L-selectin (CD26L) in naive T and B cells resulting in an impaired homing of the cells to the lymph nodes in tumor-bearing mice and therefore limiting the number of T cells responding to the presented antigen. Furthermore, they have demonstrated the ability of MDSC to induce T cell hypo-responsiveness in spleen and in blood.

CONCLUSION

In conclusion, the current study established the elevation of MDSCs in BIL and for the first time the suppressing effect of *Moringa Oleifera* therapy as a possible mechanism of action of *Moringa Oleifera* extract in rats bearing benzene-induced leukemia, thus providing a novel insight into the application of *Moringa Oleifera*-based treatments. In addition, *Moringa Oleifera* leaves extract was shown to be most promising in overcoming doxorubicin side effects when compared to doxorubicin free drug. These findings suggest that MDSCs may play a role in benzene-induced leukemia and more researches are needed to further learn about MDSCs as a potential future therapeutic target in leukemia.

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DECLARATION OF INTEREST

The authors declare that they have no conflict of interest concerning this article.

REFERENCES

1. Anand, P.; Kunnumakara, A.B.; Sundaram, C.; Harikumar, K.B.; Tharakan, S.T.; Lai, O.S.; Sung, B. and Aggarwal, B.B. (2008): Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceutical Research, 25: 2097-2116.
2. Parikh, S.A.; Jabbour, E. and Koller, C.A. (2011) Adult acute myeloid leukemia. pp. 2001-2007. *The MD Anderson Manual of Medical Oncology*. McGraw-Hill, New York, NY, City.
3. Ferrara, F. and Schiffer, C.A. (2013): Acute myeloid leukaemia in adults. The Lancet., 381: 484-495.
4. Bianchi, G.; Borgonovo, G.; Pistoia, V. and Raffaghello, L. (2011): Immunosuppressive cells and tumour microenvironment: focus on mesenchymal stem cells and myeloid derived suppressor cells. Histology and histopathology, 26: 941.
5. Marvel, D. and Gabrilovich, D.I. (2015): Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. The Journal of clinical investigation, 125: 3356.
6. Bronte, V.; Brandau, S.; Chen, S.-H.; Colombo, M.P.; Frey, A.B.; Greten, T.F.; Mandruzzato, S.; Murray, P.J.; Ochoa, A.; Ostrand-Rosenberg, S.; Rodriguez, P.C.; Sica, A.; Umansky, V.; Vonderheide, R.H. and Gabrilovich, D.I. (2016): Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nature Communications, 7: 12150.
7. Tamadaho, R.S.; Hoerauf, A. and Layland, L.E. (2017): Immunomodulatory effects of myeloid-derived suppressor cells in diseases: role in cancer and infections. Immunobiology.
8. Kumar, A.; Gautam, B.; Dubey, C. and Tripathi, P.K. (2014): A review: role of doxorubicin in treatment of cancer. International Journal of Pharmaceutical Sciences and Research, 5: 4105.
9. Carvalho, C.; Santos, R.X.; Cardoso, S.; Correia, S.; Oliveira, P.J.; Santos, M.S. and Moreira, P.I. (2009): Doxorubicin: the good, the bad and the ugly effect. Current medicinal chemistry, 16: 3267-3285.
10. Al-Asmari, A.K.; Albalawi, S.M.; Athar, M.T.; Khan, A.Q.; Al-Shahrani, H. and Islam, M. (2015): Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. PloS one., 10: e0135814.
11. Bennett, R.N.; Mellon, F.A.; Foidl, N.; Pratt, J.H.; Dupont, M.S.; Perkins, L. and Kroon, P.A. (2003): Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L.(horseradish tree) and Moringa stenopetala L. Journal of agricultural and food chemistry, 51: 3546-3553.
12. Hamza, A.A. (2010): Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats. Food and Chemical Toxicology, 48: 345-355.
13. Francis, J.A.; Jayaprakasam, B.; Olson, L.K. and Nair, M.G. (2004): Insulin secretagogues from Moringa oleifera with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. Helvetica chimica acta., 87: 317-326.
14. Mehta, K.; Balaraman, R.; Amin, A.; Bafna, P. and Gulati, O. (2003): Effect of fruits of Moringa oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. Journal of ethnopharmacology, 86: 191-195.
15. Verma, A.R.; Vijayakumar, M.; Mathela, C.S. and Rao, C.V. (2009): In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food and Chemical Toxicology, 47: 2196-2201.
16. Lipipun, V.; Kurokawa, M.; Suttisri, R.; Taweechotipatr, P.; Pramyothin, P.; Hattori, M. and Shiraki, K. (2003): Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral research, 60: 175-180.
17. Bharali, R.; Tabassum, J. and Azad, M.R.H. (2003): Chemomodulatory effect of Moringa oleifera, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pacific Journal of Cancer Prevention, 4: 131-140.
18. Abd-Rabou, A.A.; Abdalla, A.M.; Ali, N.A. and Zoheir, K.M. (2017): Moringa oleifera root induces cancer apoptosis more effectively than leave nanocomposites and its free counterpart. Asian Pacific journal of cancer prevention: APJCP., 18: 2141.
19. Jung, I.L. (2014): Soluble extract from Moringa oleifera leaves with a new anticancer activity. PloS one., 9: e95492.
20. Olufemi, A.E.; Terry, A.O. and Kola, O.J. (2012): Anti-leukemic and immunomodulatory effects of

fungal metabolites of Pleurotus pulmonarius and Pleurotus ostreatus on benzene-induced leukemia in Wister rats. The Korean journal of hematology, 47: 67-73.
21. El-Mehy, A.E. (2008): Histological And Immunohistochemical Study On The Effect Of Doxorubicin On The Heart Of Adult Albino Rat And The Possible Protective Role Of An Antioxidant Abeer E. El-Mehy; Fouad K. Mansour; Nariman A. Abd El-Fattah; Fatma A. El-Safty and Mohamed M. El-Fiky. MMJ, Jan 2008; 21: 91-108.
22. Radwan, H.A.; Ghaly, I.S.; Farag, I.M. and Ezzo, M. (2015): Protective and Therapeutic Effect of Moringa oleifera Leaf Extract on DNA Damage, Cytogenetic Changes, Sperm Abnormalities and High Level of MDA Induced by CCL4 in Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6.
23. Papież, M.A.; Krzyściak, W.; Szade, K.; Bukowska-Straková, K.; Kozakowska, M.; Hajduk, K.; Bystrowska, B.; Dulak, J. and Jozkowicz, A. (2016): Curcumin enhances the cytogenotoxic effect of etoposide in leukemia cells through induction of reactive oxygen species. Drug design, development and therapy, 10: 557.
24. Sheta, A.; Elsakkar, M.; Hamza, M. and Solaiman, A. (2016): Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in adult male albino rats. Human & experimental toxicology, 35: 1227-1239.
25. Kulkarni, J. and Swamy, A.V. (2015): Cardioprotective effect of gallic acid against doxorubicin-induced myocardial toxicity in albino rats. Indian Journal of Health Sciences and Biomedical Research (KLEU)., 8: 28.
26. Thirumaran, R.; Prendergast, G.C. and Gilman, P.B. (2007) Chapter 7 - Cytotoxic Chemotherapy in Clinical Treatment of Cancer. pp. 101-116. *Cancer Immunotherapy*. Academic Press, City.
27. Osman, H.M.; Shayoub, M.E. and Babiker, E.M. (2012): The effect of Moringa oleifera leaves on blood parameters and body weights of albino rats and rabbits. Jordan Journal of Biological Sciences, 5: 147-150.
28. Alabi, O.; Malik, A.; Ng'ambi, J.; Obaje, P. and Ojo, B. (2017): Effect of Aqueous Moringa Oleifera (Lam) Leaf Extracts on Growth Performance and Carcass Characteristics of Hubbard Broiler Chicken. Revista Brasileira de Ciência Avícola., 19: 273-280.
29. Furo, N. and Ambali, A. (2013): An investigation into the phytochemical constituents of Moringa oleifera aqueous root extracts. Centrepoint Journal (Science Edition). 18.
30. Anwar, F.; Latif, S.; Ashraf, M. and Gilani, A.H. (2007): Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy research, 21: 17-25.
31. 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. Journal of agricultural and food chemistry, 51: 2144-2155.
32. Fadi, C.; Andrzj, P.; Ekaterina, M. and Hayes, K. (2010): Nutrition correlates and dynamics of diabetes in Nile rats: a novel model for diet induced type 2 diabetes and metabolic syndrome. J Nutri.
33. Khan, I.; Zaneb, H.; Masood, S.; Yousaf, M.; Rehman, H. and Rehman, H. (2017): Effect of Moringa oleifera leaf powder supplementation on growth performance and intestinal morphology in broiler chickens. Journal of animal physiology and animal nutrition, 101: 114-121.
34. Tacar, O.; Sriamornsak, P. and Dass, C.R. (2013): Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. Journal of Pharmacy and Pharmacology, 65: 157-170.
35. Ashley, N. and Poulton, J. (2009): Mitochondrial DNA is a direct target of anti-cancer anthracycline drugs. Biochemical and biophysical research communications, 378: 450-455.
36. Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G. and Gianni, L. (2004): Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacological reviews, 56: 185-229.
37. Leung, L.K. and Wang, T.T. (1999): Differential effects of chemotherapeutic agents on the Bcl-2/Bax apoptosis pathway in human breast cancer cell line MCF-7. Breast cancer research and treatment, 55: 73-83.
38. Srivastava, J.K. and Gupta, S. (2006): Tocotrienol-rich fraction of palm oil induces cell cycle arrest and apoptosis selectively in human prostate cancer cells. Biochemical and biophysical research communications, 346: 447-453.
39. Lyu, Y.L. and Liu, L.F. (2012) 13 - Doxorubicin Cardiotoxicity Revisited: ROS Versus Top2. pp. 351-369. *Recent Advances in Cancer Research and Therapy*. Elsevier, City.
40. Sathya, T.; Aadarsh, P.; Deepa, V. and Murthy, P.B. (2010): Moringa oleifera Lam. leaves prevent cyclophosphamide-induced micronucleus and DNA damage in mice. International Journal of phytomedicine, 2.
41. Devaraj, S.; Mathur, S.; Basu, A.; Aung, H.H.; Vasu, V.T.; Meyers, S. and Jialal, I. (2008): A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. Journal of the American College of Nutrition, 27: 267-273.
42. Berkovich, L.; Earon, G.; Ron, I.; Rimmon, A.; Vexler, A. and Lev-Ari, S. (2013): Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC complementary and alternative medicine, 13: 212.
43. Charoensin, S. (2014): Antioxidant and anticancer activities of Moringa oleifera leaves. Journal of Medicinal Plants Research, 8: 318-325.

44. Akanni, O.E.; Adedeji, A.L. and Oloke, K.J. (2014): Abstract 3792: Upregulation of TNF-α by ethanol extract of Moringa oleifera leaves in benzene-induced leukemic Wister rat: a possible mechanism of anticancer property. Cancer Research, 74: 3792-3792.
45. Abdou, H.; SH, S.; Booles, H.F. and EA, A.R. (2012): Effect of pomegranate pretreatment on genotoxicity and hepatotoxicity induced by carbon tetrachloride (CCl4) in male rats. Journal of Medicinal Plants Research, 6: 3370-3380.
46. ACKLAND, M.L.; VAN DE WAARSENBURG, S. and Jones, R. (2005): Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. In vivo., 19: 69-76.
47. Tragulpakseerojn, J.; Yuki, R.; Honda, T.; Morii, M.; Apirakaramwong, A.; Yamaguchi, N.; Pamonsinlapatham, P. and Yamaguchi, N. (2014): Apoptotic activities of the extract from Moringa oleifera leaves on human HCT116 colon cancer cells. Fundamental Toxicological Sciences, 1: 143-149.
48. Dolen, Y.; Gunaydin, G.; Esendagli, G. and Guc, D. (2015): Granulocytic subset of myeloid derived suppressor cells in rats with mammary carcinoma. Cellular immunology, 295: 29-35.
49. Shen, P.; Wang, A.; He, M.; Wang, Q. and Zheng, S. (2014): Increased circulating Lin−/lowCD33+ HLA-DR− myeloid-derived suppressor cells in hepatocellular carcinoma patients. Hepatology Research, 44: 639-650.
50. Giallongo, C.; Parrinello, N.; Tibullo, D.; La Cava, P.; Romano, A.; Chiarenza, A.; Barbagallo, I.; Palumbo, G.A.; Stagno, F.; Vigneri, P. and Di Raimondo, F. (2014): Myeloid derived suppressor cells (MDSCs) are increased and exert immunosuppressive activity together with polymorphonuclear leukocytes (PMNs) in chronic myeloid leukemia patients. PLoS One., 9: e101848.
51. Zhang, B.; Wang, Z.; Wu, L.; Zhang, M.; Li, W.; Ding, J.; Zhu, J.; Wei, H. and Zhao, K. (2013): Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PloS one., 8: e57114.
52. Van Valckenborgh, E.; Schouppe, E.; Movahedi, K.; De Bruyne, E.; Menu, E.; De Baetselier, P.; Vanderkerken, K. and Van Ginderachter, J. (2012): Multiple myeloma induces the immunosuppressive capacity of distinct myeloid-derived suppressor cell subpopulations in the bone marrow. Leukemia., 26: 2424.
53. Ostrand-Rosenberg, S. and Sinha, P. (2009): Myeloid-derived suppressor cells: linking inflammation and cancer. The Journal of Immunology, 182: 4499-4506.
54. Rodríguez, P.C. and Ochoa, A.C. (2008): Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunological reviews, 222: 180-191.
55. Sauer, H.; Wartenberg, M. and Hescheler, J. (2001): Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, 11: 173-186.
56. Gabrilovich, D.I. and Nagaraj, S. (2009): Myeloid-derived suppressor cells as regulators of the immune system. Nature reviews immunology, 9: 162-174.
57. Serafini, P.; Mgebroff, S.; Noonan, K. and Borrello, I. (2008): Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer research, 68: 5439-5449.
58. Ku, A.W.; Muhitch, J.B.; Powers, C.A.; Diehl, M.G.; Kim, M.; Fisher, D.T.; Sharda, A.P.; Clements, V.K.; O'Loughlin, K. and Minderman, H. (2016): Tumor-induced MDSC act via remote control to inhibit L-selectin-dependent adaptive immunity in lymph nodes. Elife., 5: e17375.