Biochemical effect of Moringa peregrina Seeds on experimentally leukcytopenia in Rats

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ABSTRACT

The present study was to evaluate anti-leukopenia and antioxidant effects of Moringa peregrina seeds oil at dose of 381 mg/kg in 400mg/kg.b.w Benzene and/or 20 mg/kg.b.w 5-Flourourasil induced leukopenia rats 2 weeks after induction of leukopenia in rats, Moringa peregrina seeds oil at dose of 381 mg/kg was administrated for 30 consecutive days. On the 31th day, the rats were sacrificed for the estimation of Hemoglobin (Hb%), Total Leucocyte Count (WBC) and Platelet Count (PLT) as well as biochemical parameters; Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Albumin(Alb) , Total Protein, Lactate Dehydrogenase (LDH), Creatine Kinase (C.K), Urea , Creatinine ,Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Total Cholesterol (T.Ch), Triglycerides (TG), P_{53} Gene Expression, Nitric Oxide (NO) , Thiobarbituric Acid Reactive Substances (TBARs) and Tumor Necroses Factor-α (TNF-α). The results of this study also showed that administration of Moringa peregrina seeds oil at dose of 381 mg/kg.b.w to leukopenia induced rats demonstrated a significant (P<0.01) increase in Hb%, WBCs and PLT as well as a significant (P<0.01) improvement in biochemical parameters and life span as compared to the benzene and /or 5-Flourourasil control rats. The histological examinations of this study revealed lung damage and degeneration in the lung of benzene and /or 5-Flourourasil treated rats. Also, lung of Moringa peregrina seeds oil at 381mg/kg rats showed significant improvement and protection against benzene and /or 5-Flourourasil harmful effect. On the other hand, the results clearly suggest that the oxidative stress of benzene higher than 5-Flourourasil

Key words: Moringa seeds oil, 5-Flourourasil, benzene, leukopenia and antioxidants.

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1. INTRODUCTION

Leucopenia clinical condition characterized by an absolute reduction in circulating white blood cells below the lower limit of normal values. The White blood cells are basically the soldiers of the body responsible for fighting away all infections and foreign agents. They are responsible for protecting the body against all microorganisms. The level of leucocytes can fall due to many reasons ranging from malignancies, the disease of the bone marrow, any severe infections and autoimmune disorders, chemotherapy, radiation therapy, exposure to drugs &chemicals like benzene, certain biological therapies such as interleukin-2 (Aldesleukin, Proleukin) or rituximab (Rituxan) and there can be many other causes of leucopenia (Lee GR, et al, 1999). Human exposure to benzene occurs primarily via inhalation in the workplace, from gasoline vapors, tobacco smoke, and automotive emissions. Individuals exposed to benzene exhibit bone marrow depression, as evidenced by anemia (decreased RBC count), leukopenia (decreased WBC count),

and/or thrombocytopenia (decreased platelet count). A depression of all three elements is called pancytopenia, and the simultaneous depression of RBCs, WBCs, and Platelets, accompanied by necrosis of the bone marrow, is diagnostic of aplastic anemia. Patients with aplastic anemia also have exhibited mild bilirubinemia, changes in osmotic fragility of erythrocytes, shortened erythrocyte survival time, increased fecal urobilinogen, and mild reticulocytosis (Aksoy, 1991). 5-Fluorouracil (5-FU) is an antimetabolite that acts during
The S phase of the cell cycle the toxicity of 5-FU, which includes leukopenia, diarrhea, stomatitis, nausea, vomiting, and alopecia, differs with its schedule of administration (Mates et al., 1999). Plants vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discovery and development (Sobrero et al., 1997& Ebenezer et al., 2011). It is widely accepted that a diet rich in fruits and plants are rich sources of different kinds of antioxidants, phenolic compounds are the most studied and have been recognized to possess a wide range of properties including antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticarcinogenic actions (Sobrero et al., 1997). Many of the biological functions of flavonoids, phenolic, catechins, curcumin, resveratrol and genistein compounds have been attributed to their free radical scavenging, metal ion chelating and antioxidant activities (Ebenezer et al., 2011&Seef et al., 2011). Several medicinal plants have been implicated in the mechanisms of chemoprevention which refers to the use chemical substances of natural origin or synthetic to reverse, retard or delay the multistage carcinogenesis process (Ebenezer et al., 2011).One of such plants, Moringa peregrina ranks high among seeds in both antioxidant quality and quantity (Koheil et al., 2011) .Because of its substantial flavonoids content and a wealth of phenolic acids. Moringa peregrina tree belongs to the Moringaceae family, commonly known as a drumstick tree that is native to tropical widely naturalized and cultivated in many countries including Malaysia (Okuda et al., 2001). A literature survey indicated that the presence of quercetin, flavonoids (Selvakumar& Natarajan, 2008), sterols (Yammuenart et al., 2008), tocopherols (γ and α), β-carotene and other antioxidants (Anwar et al., 2007) have been reported from the plant. The different extracts of the plant were also screened for In vitro anti-inflammatory and antioxidant activities (Koheil et al., 2011) as an extension of my interested research program in the extraction and therapeutic evaluation of rare medicinal plants (Hussein, 2008; Hussein, 2010; Hussein and Abdelgwade, 2010). We report herein, a facile route to explain antioxidant of Moringa peregrina seeds oil against benzene and/or 5-flurouracil induced leukopenia in female albino rats.

2. MATERIALS AND METHODS

2.1. Chemicals:

5-fluorouracil and Benzene were from Merck Ltd., Germany. All the other reagents used were of analytical grade and were obtained commercially.

2.2. Induction of Leukopenia

Leukopenia has been demonstrated to occur in rats by oral administration of 400 mg/kg.b.w benzene and/or I.P injection of 20mg/kg.b.w. 5-Flourourasil day after day for 2 weeks (IARC, 1982 and Shun et al., 1996).

2.3. Plant material:

Moringa peregrina seeds were obtained from Ankit Agrowal, India. The plant material was identified, authenticated taxonomically by Dr. Heba El-Gezawy, Pharmacognosy department, faculty of Pharmacy, October 6 University. The seed were cleaned, dried under direct sunlight and powdered by a mechanical grinder.

2.4. Extraction of Fixed oil:

After being cleaned by hand carefully to remove the foreign materials such as other seeds, stones and small stalks, Moringa peregrina seed were dried at 50°C for 12h in an oven, and then crushed into powder in a grinder with a size range of 0.55-1.0 mm. The resulted powder was kept in a vacuum dryer until use. Moringa peregrina ground samples were mixed with hexane (1:10, m/V) at (60-80°C) using a Soxhlet apparatus. This process of extraction was repeated for 6h, the hexane distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature of 40±2 °C.

2.5. Rats

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6University. Adult rats weighing around 180 ± 20 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2ºC, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet adlibitum.

2.6. Experimental design:

The animals were divided into 5 groups consisting of 8 animals, three controls groups and two treatment groups: Group (1): Negative control. Group (2): Positive control-A (400mg/kg.b.w. benzene, orally). Group (3): Positive control-B (20mg/kg.b.w. 5-fluorouracil, I.P). Group (4): Leukopenia bearing rats (400mg/kg.b.w. benzene, orally) +1/30 LD_{50} (381mg/kg.b.w Moringa peregrina seeds oil daily for 4 weeks. Group (5): Leukopenia bearing rats (20mg/kg.b.w.5-fluorouracil, I.P) + 1/30 LD_{50} (381 mg/kg.b.w. Moringa peregrina seeds oil daily for 4weeks. On 31th day, at the end of the study, all rats were sacrificed blood was collected one part of blood was collected for hematological parameters such as Hemoglobin (Hb), Total Leucocyte count (WBC) and Platelet count (PLT) were determined as described by Jain, (1986). In addition, the other part centrifuged, and plasma was used freshly for estimation of plasma Transaminase (L-alanine and L-aspartate) (Reitman and Frankel, 1957), Alkaline phosphatase (ALP) (Kind and King, 1954). Also, Lactate Dehydrogenase (LDH) (Buhl and Jackson, 1978), TBARS, Nitric Oxide (NO), Tumor Necrosis Factor-α (TNF-α) and GSH levels in blood and hepatic were done by the methods described by Nichansand Samulelson (1968), Miranda et al., (2001), Beyaert and Fiers(1998)and Chanarin (1989), respectively. Blood and lung Superoxide dismutase (SOD) and catalase (CAT) activities were carried out by Marklund S, Marklund (1974), Sinha (1972), respectively. Plasma triglyceride and total cholesterol were determined using commercially available kits (Fossati P, Prencipe (1982), Allain et al., 1974 and Friedewald, 1972). Finally, lung p53gene were determined according to the method described by Tribukait (1984).

2.7. Determination of lung P53 gene

2.7.1. Primer Design

Primers were designed based on the genomic and mRNA sequences retrieved from gene sequence databases such as National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The location of every intron and exon was determined within the gene sequences based on the mRNA sequence in order to design the primers at exon-exon junctions to avoid the false positive results arising from amplification of possible contaminating genomic DNA. Primers were checked by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure that they did not have any non-specific binding sites on either the same gene or similar sequence sites in other species. The primer sequences was as follow:

Official name	Forward Primer	Reverse Primer
Beta Actin	5′ CGTGCGTGACATTAAAGAGAA 3′	5′ CGCTCATTGCCGATAGTGAT 3′
P53	5′ GGAGAATATTTCACCCTTAAGATCC 3′	5′ GAGTGAGCCCTGCTGTCTCCT 3′

2.7.2. Gene-specific PCR for amplification of the P53 gene

PCR was performed using GoTaq Green Master Mix (Promega, Madison, US) in order to set up the optimum annealing temperature for P53 gene. A 25µl PCR reaction was set up as follows: 12.5ul of 2X GoTaq Green Master Mix, which contains GoTaq® DNA Polymerase that is supplied in 2X Green GoTaq® Reaction Buffer (pH 8.5), 400 µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl2; 400nM of 10uM, 200ng cDNA template, and appropriate volume of nuclease free water was added to a 0.2ml nuclease free PCR tubes and centrifuged for 10sec. The thermal cycler was adjusted as follows: initial denaturation step at 95°C for 2min, subsequent denaturation step at 95°C for 30s, optimization of the annealing conditions by performing the gradient reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 3°C to the annealing temperature for 30sec, followed by 72°C for 25sec for template extension and a final extension of 5 minutes at 72°C, following a 4°C incubation for 10min. All the PCR reaction preparation steps were performed on ice.

2.7.3. Agarose Gel Electrophoresis

The GoTaq® Reaction Buffer contains yellow and blue dyes, the blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers (<50bp), in a 1% Agarose gel. The PCR product was separated on 2% Agarose gel containing 0.1% ethidium bromide (EtBr) and the DNA bands were visualized with a UV transilluminator containing an EtBr filter. The image system was a Gel Doc EZ System imager.

2.7.4. Analysis of the Relative Expression level of the P53 gene:

The relative levels of expression of each gene were analyzed by taking the band intensity using Quantity One software, Biorad. The ratios of desired genes/β-actin product were subsequently calculated after subtraction of the background pixel intensity for each gene of interest and used to assess the differences in expression levels between control and different groups.

2.8. Histological assessment:

Lungs from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin Eosin (H&E) for light microscopic analyses according to the method of Bancroft and Steven (Bancroft and Steven, 1983). The slides were coded and were examined by a histopathologist who was ignorant about the treatment groups after which photographs were taken.

2.9. Statistical analysis

All data were expressed as mean ± SE. All analyses utilized SPSS 13.0 statistical package for Windows (SPSS, 13.0 software, Inc., Chicago, IL, 2009) (2012). A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value 0.05 was accepted as statistically significant. Benzene and /or 5-fluorouracil administrated positive control rats were compared with normal control rats as well as experimental groups (Moringa peregrina oil extract + benzene and/or 5-fluorouraciltreated rats were compared with positive controls A &B.

3. RESULTS

Oral administration of benzene at 400 mg/kg.b.w and/or 20 mg/kg.b.w 5-fluorouracil resulted in a significant decrease in blood hemoglobin, complete blood cell count Leukocyte (WBC) and platelet count (PLT) compared to the normal control group (p<0.01). Group of rats received benzene at 400mg/kg.b.w. and/or 20 mg/kg.b.w 5-fluorouracil and treated with Moringa peregrina seeds oil at 381mg/kg. Resulted in a significant increase in hemoglobin, WBCs and PLT compared to the positive controls A and B respectively (p<0.01) (Table 1).

Table 2 showed that oral administration of benzene and/or 5-fluorouracil resulted in a significant increase in plasma AST, ALT and ALP (p<0.01) as well as non-significant change in plasma albumin and total protein compared to the normal control group. Administration of Moringa peregrina seeds oil at 381mg/kg.b.w to rats resulted in a significant decrease in plasma AST, ALT and ALP (p<0.01) as well as non-significant change in plasma albumin and total protein compared to the positive controls A and B respectively. Table 3 showed that oral administration of benzene and/or 5-fluorouracil resulted in a significant increase in plasma lactate dehydrogenase (LDH) and Creatine kinase (CK) compared to the normal control group (p< 0.01). Administration of Moringa peregrina seeds oil at 381mg/kg.b.w to rats resulted in a significant decrease in plasma LDH and CK compared to the positive controls A and B respectively (p<0.01). Table 4showed that oral administration of benzene and/or 5-fluorouracil resulted in a significant increase in plasma urea and creatinine compared to the normal control group (p< 0.01). Administration of Moringa peregrina seeds oil at 381mg/kg.b.w to rats resulted in a significant decrease in plasma urea and creatinine compared to the positive controls A and B respectively (p<0.01). Tables 5& 6showed that oral administration of benzene and/or 5-fluorouracil resulted in a significant increase in blood and lung TBARs as well as decrease GSH, SOD and CAT levels compared to the normal control group (p< 0.01). Administration of Moringa peregrina seeds oil at 381mg/kg.b.w to rats resulted in a significant decrease in blood and lung TBARs as well as increase in GSH, SOD and CAT levels compared to the positive controls A and B respectively (p<0.01). Table 7 showed that oral administration of 400mg benzene and/or 20mg/kg/b/w. 5-fluorouracil resulted in a significant increase in plasma cholesterol (TCh) and triglycerides (TG) compared to the normal control group (p< 0.01). Administration of Moringa peregrina seeds oil at 381mg/kg. to rats resulted in a significant decrease in plasma TCh and TG compared to the positive controls A and B respectively (p<0.01).Tables 8 showed that oral administration of 400mg benzene and/or 20mg/kg/b/w. 5-fluorouracil resulted in a significant increase in plasma and lung nitrous oxide (NO) and tumor necrosis factor-α (TNF-α) compared to the normal control group (p<0.01). Administration of Moringa peregrina seeds oil at 381mg/kg. to rats resulted in a significant decrease in plasma and lung nitrous oxide (NO) and tumor necrosis factor-α (TNF-α) compared to the positive controls A and B respectively (p<0.01).Table 9 showed that oral administration of 400mg benzene and/or 20mg/kg/b/w. 5-fluorouracil resulted in a significant decrease in plasma T_3, $T_4$and as well as significant increase in plasma TSH compared to the normal control group (p< 0.01). Administration of Moringa peregrina seeds oil at 381mg/kg. to rats resulted in a non-significant change in plasma T_3 and significant increase in plasma T_4 as significant decrease in plasma TSH compared to the positive controls A and B respectively (p<0.01).

Reverse transcriptase PCR (Figure 1 and chart 1) results shown that lung *P53* mRNA expression level was significant increased (p< 0.01) in benzene and/or 5-fluorouracil treated groups into 215±22.5 and 162±8.9,

respectively when compared to negative control group (100±5.2). However, reverse transcriptase PCR results shown that *P53* mRNA expression level was significant decreased ($p< 0.05$) in Moringa peregrina seeds oil treated group (150±15.4) and (198±23.3) when compared to benzene and/or 5-fluorouracil positive controls A and B, respectively.

Table 1: Effect Moringa peregrina seeds oil (MPO), 5-fluorouracil and there combination on hematological parameters in benzene induce Leukopenia in rats

No.	Groups	Hb% (g/dL)	WBCs ($x10^3$/ mm^3)	PLT 10^3/ mm^3
(I)	Normal group	12.15 ± 1.45	9.7 ± 0.65	506 ± 7.50
(II)	Control positive –A (400mg/kg.b.w. benzene) Control positive – B (20mg/k.g. 5-flurouracil)	11.9 ± 0.87@	3.8 ± 0.90*	433 ± 5.60*
(III)	Control positive –A (400 mg/kg.b.w. benzene) + 381mg/kg.w.b. Moringa peregrina seeds oil	11.46 ± 0.68*	4.10 ± 0.84*	457 ± 6.90*
(IV)	Control positive – B (20mg/k.g. 5-flurouracil) + 381mg/kg.w.b. Moringa peregrina seeds oil	12.75 ± 1.60@	7.00 ± 0.50@	525± 13.80@
(V)		13.65± 1.20@	7.60 ± 1.10@	610 ± 11.00@

5-Flourourasil was given i.p as a daily dose of 20mg/kg b.w.381mg/kg.w.b. Moringa peregrina seeds oil was orally given daily for 4weeks. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

Table 2: Effect of Moringa peregrina seeds oil on plasma Alanine transaminase (ALT), Aspartate transaminase (AST), alkaline phosphatase (ALP), albumin and total protein levels in normal and experimental groups of rats

Groups	(I)	(II)	(III)	(IV)	(V)
Total protein (g/dl)	7.23± 0.70	7.36± 0.57	7.63± 0.45	7.43± 0.64	7.46± 0.93
Albumin (g/dl)	3.56± 0.54	3.38± 0.35	3.67± 0.54	3.65± 0.40	3.68± 0.51
ALP (U/L)	80.67± 5.47	184.16± 14.65*	210.76± 15.87*	115.76±15.43@	93.08± 6.55@
AST (U/L)	16.39± 1/55	29.43± 4.20*	30.90± 3.09*	19.98± 3.11@	17.60± 3.40@
ALT (U/L)	12.5± 2.30	24.60± 2.46*	35.46± 4.88*	18.57± 2.89@	11.30± 2.00@

Table 3: Effect of Moringa peregrina seeds oil on plasma lactate dehydrogenase (LDH) and Creatine Kinase (CK) levels in normal and experimental groups of rats

No.	Groups	LDH (U/L)	CK (mU/L)
(I)	Normal group	157.1± 10.90*	239.33± 18.72*
(II)	Control positive –A (400mg/kg.b.w. benzene)	210.66± 8.75*	346.83± 10.90*
(III)	Control positive – B (20mg/k.g. 5-flurouracil)	258.9± 21.90*	607.66± 22.45*
(IV)	Control positive –A (400 mg/kg.b.w. benzene) + 381mg/kg.w.b. Moringa peregrina seeds oil	169.50± 13.50*	266.66± 10.88*
(V)	Control positive – B (20mg/k.g. 5-flurouracil) + 381mg/kg.w.b. Moringa peregrina seeds oil	149.00± 16.00*	222.11± 11.20*

Table 4: Effect of Moringa peregrina seeds oil on plasma urea and creatinine levels in normal and experimental groups of rats

No.	Groups	Urea (mg/dl)	Creatinine (mg/dl)
(I)	Normal group	44.38±5.40*	0.94±0.08*
(II)	Control positive –A (400mg/kg.b.w. benzene)	61.46± 3.25*	1.98±0.24*
(III)	Control positive – B (20mg/k.g. 5-flurouracil)	50.77± 6.87*	1.84±0.17*
(IV)	Control positive –A (400 mg/kg.b.w. benzene) + 381mg/kg.w.b. Moringa peregrina seeds oil	39.21± 4.66*	0.93±0.08*
(V)	Control positive – B (20mg/k.g. 5-flurouracil) + 381mg/kg.w.b. Moringa peregrina seeds oil	38.53± 3.25*	0.88±0.054*

Table 5: Effect of Moringa peregrina seeds oil on blood Reduced Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) and Thiobarbituric acid reactive substances (TBARs) levels in normal and experimental groups of rats

Groups	(I)	(II)	(III)	(IV)	(V)
TBARs (μmol/ml)	3.54± 0.81	6.88± 0.57*	5.49± .50*	3.93 ± 0.39	3.62 ± 0.43
CAT (U/ml)	35.22± 2.25	20.23± 2.86*	22.52± 1.27*	27.33 ±1.08*	31.32± 0.98*
SOD (U/ml)	251.30± 16.54	132.71±8.44*	177.78±13.28*	213.86± 2.51*	228.95± 3.56*
GSH (mg %)	33.44±2.85	11.36± 1.64*	17.95± 2.25@	24.55± 3.23*	28.48± 2.04*

Table 6. Effect of Moringa peregrina seeds oil on blood Reduced Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT) and Thiobarbituric acid reactive substances (TBARs) levels in normal and experimental groups of rats

Groups	(I)	(II)	(III)	(IV)	(V)
TBARs (mg/100g tissue)	0.79± 0.11	1.83± 0.08*	1.43± 0.0.14*	0.77±0.06*	0.71± 0.04*
CAT (Umol H_2O_2 consume/mg tissue)	48.58± 5.30	17.48± 2.84*	16.47± 3.60*	31.73± 0.84*	36.08 ±1.45*
SOD (U/100gm tissue)	345.24± 14.56	150.54±10.30*	202.22±16.49*	302.24± 4.13*	330.29± 3.99*
GSH (mg/100g tissues)	145.61± 12.44	68.37 ± 8.72*	81.25 ± 6.97@	114.82 ±2.60*	125.84 ± 2.58*

Table 7: Effect of Moringa peregrina seeds oil on plasma total cholesterol (TCh) and triglycerides (TG) levels of normal and experimental groups of rats

No.	Groups	TCh (mg/dl)	TG (mg/dl)
(I)	Normal group	117.39± 5.47	98.09± 7.00
(II)	Control positive –A (400mg/kg.b.w. benzene)	287.50±22.74*	239.70±11.80*
(III)	Control positive – B (20mg/k.g. 5-flurouracil)	246.23±21.4@	220.00±13.75@
(IV)	Control positive –A (400 mg/kg.b.w. benzene) + 381mg/kg.w.b. Moringa peregrina seeds oil	210.45±11.4@	150.98±11.20@
(V)	Control positive – B (20mg/k.g. 5-flurouracil) + 381mg/kg.w.b. Moringa peregrina seeds oil	157.60±7.35@	121.39±10.74@

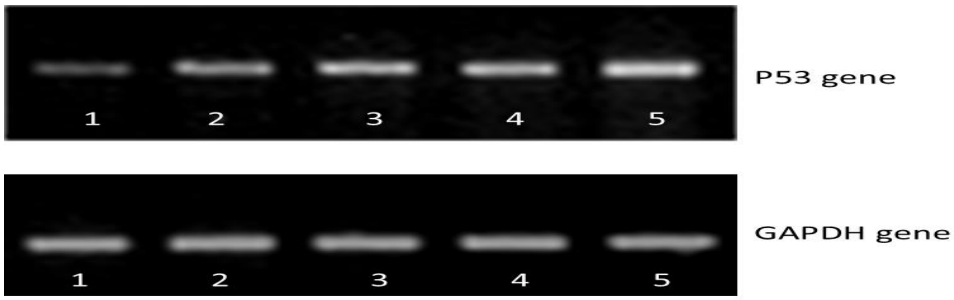
Table 8: Effect of Moringa peregrina seeds oil on plasma and lung Nitrous Oxide (NO) as well as Tumor Necrosis factor-α (TNF-α) levels of normal and experimental groups of rats

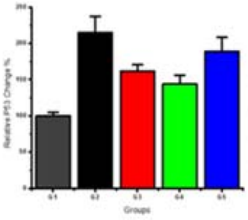
	NO		TNF-α	
Groups	Plasma (umol/m)	Lung (u mol/g tissue	Plasma U/ml	Lung (u mol/g tissue
1	42.88 ± 4.35	66.93 ± 2.25	237.55 ± 11.25	15.17 ± 1.07
2	57.86 ± 5.40*	98.91 ± 7.46*	342.39 ±10.87*	33.36 ± 3.28*
3	44.83 ± 2.87@	84.14± 2.76@	301.74 ± 18.58@	32.13 ± 4.66@
4	38.45 ± 5.07*	54.01±4.04*	265.74± 20.70*	24.51±3.11*
5	31.64±3.40*	50.33±6.37*	243.41± 19.65*	20.54± 2.76*

Table 9: Effect of Moringa peregrina seeds oil on plasma Total Triiodothyronine (T3), Thyroxin (T4) and Thyroid Stimulating Hormone (TSH) levels of normal and experimental groups of rats

Groups No.	T_3 (ng/dl)	T_4 (ng/dl)	TSH (ng/dl)
(I)	1.52± 0.32	6.15± 0.5	0.7± 0.013
(II)	0.69± 0.015*	3.29± 0.67*	0.91± 0.006*
(III)	0.61± 0.032*	3.15± 0.34*	0.96± 0.14*
(IV)	0.72± 0.034	4.09@± 0.54	0.67± 0.008
(V)	0.71± 0.083	3.32± 0.40@	0.77±0.009@

Figure 1: Reverse transcriptase PCR of lung*P53* mRNA expression level





G1: Negative control
G2: Positive control-A (400mg/kg.b.w. Benzene)
G3: Positive control-B (20mg/kg.b.w. 5-fluorouracil)
G4: Positive control-A (400mg/kg.b.w. Benzene) + 381mg/kg.b.w. Moringa peregrina seeds oil.
G5: Positive control-A (20mg/kg.b.w. 5-fluorouracil) + 381mg/kg.b.w. Moringa peregrina seeds oil.

Chart (1):Analysis of lung P53 transcript by semi-quantitative PCR in lung tissues A) P53 amplicon B) GAPDH amplicon from Control Negative (1), (control positive-A; benzene treated group (2), control positive-B (5-fluorouracil treated group)(3), (control positive-A + Moringa peregrina seeds oil) (4) and (control positive-B+ Moringa peregrina seeds oil treated group) (5).

4. DISCUSSION

The present article aimed to study the antitumor activity of administration of Moringa peregrina seeds oil at 381mg/kg. In benzene and/or 5-flurouracil induce Leukopenia in rats. Our results confirmed with Aksoy, 1991 and Sobrero et al., 1997 which showed that benzene and 5-flurouracil. The anti-Leukopenia effect of Moringa peregrina seeds oil was investigated using leucopenia induced rat model, when injected with benzene and/or 5-flurouracil by 2-weeks Aksoy, 1991 and Sobrero et al., 1997. In this study, we observed and reported that Moringa peregrina seeds oil can normalize the levels of hematological parameters, which may be due to the presence of antioxidant phytochemicals (Hussein, 2010 and Koheil et al., 2011). Liver is considered to be the main organ of drug detoxifying organ, some lung marker enzyme levels were measured from plasma. AST, ALT, ALP, LDH, NO, α -TNF and TBARs levels were increased in benzene and/or 5-flurouracil induced leukopenia groups, whereas GSH, SOD and CAT levels were decreased in blood and lung tissue. Reactive oxygen species (ROS), like superoxide anions, are under normal physiological conditions cleared by antioxidant defense system such as GSH, SOD and CAT. Superoxide anion is dismutated to hydrogen peroxide (H_2O_2) in a process catalyzed by SOD, and $H_2O_2$is then eliminated by catalase or GSH-Px

(Sterba et al., 2013). The activities of SOD and GSH-Px were lowered in 5-FU treated guinea pigs (Durak et al., 2000) demonstrating a reduced antioxidant capacity. If not eliminated by cellular antioxidant systems, superoxide anions can generate the highly reactive and toxic hydroxyl radicals (−OH) through the Haber–Weiss reaction, which is catalyzed by iron (Kehrer, 2000). Increased ROS levels inside cells lead to oxidation of macromolecules ,including lipids, nucleic acids, and proteins, thereby disturbing cellular functions (Kehrer, 2000). MDA is a frequently used marker of lipid peroxidation (Nielsen et al., 1997),and MDA levels were elevated in guinea pig hearts after 5-FU-treatment (Durak et al., 2000), and slightly elevated (but not significantly) in isolated rat hearts after 5-FU-treatment (Millart et al., 1993). These findings indicate that some degree of oxidative stress and cellular damage takes place in animal hearts during 5-FU-treatment. Our study suggested that 5-FU-induceddamage to the arterial endothelium may be due to generation of free radicals, resulting in lipid peroxidation (Asirvatham and Christina 2012). Phanolic compounds of Moringa peregrina seeds oil increases SOD and CAT activities in animals, hereby improving antioxidant potential (Hussein, 2010 and Koheil et al., 2011). In the present study, administration of benzene and/or 5-flurouracil resulted in a significant decrease in blood GSH, SOD and CAT as well as plasma TC, TG, HDL-C and LDL-C with a significant increase in plasma TBARs compared to the normal control group. These results were in agreement with Gupta et al., (2007) and Raju et al., (2012) who reported that the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity (Abu-Sinna et al., 2003). On treatment with Moringa peregrina seeds oil altered lung enzyme level was restored as that of the normal group. Alterations of cholesterol metabolism, including increased cholesterol synthesis and accumulation of cholesterol esters in tumor tissues associated with a decrease of high-density lipoprotein cholesterol in serum, were previously observed indifferent models of neoplastic cell proliferation including hematological malignancies. A number of studies had indicated that reactive oxygen species (ROS) are involved in a variety of different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis.

Flavonoids and other phenolic compounds are well known natural antioxidants. The flavonoids present in Moringa peregrina seeds oil are thought to be the cause of their anti tumor and anti-inflammatory effects (Hussein, 2010 and Koheil et al., 2011). Flavonoids have a chemo preventive role in cancer by means of their effect in signal transduction in cell proliferation and angiogenesis (Wagner et al., 1986). This important property may be responsible for its Leukopenia activity against benzene and/or 5-flurourail. Antioxidant activity of Moringa peregrina seeds oil against different reactive oxygen and nitrogen species as already been established by the present authors (Hussein, 2010 and Koheil et al., 2011). The present work showed that benzene and/or 5-flurouracil administration caused increase of lung *P53* mRNA expression level when compare with normal control rats.Furthermore, Moringa peregrina seeds oil induces apoptosis in p53-null lung cancer cells. Moringa peregrina seeds oilcan block cell cycle progression or even apoptosis in a p53-independent manner as well, especially in the cells that lack functional p53 (Nicholson et al., 1995 and Chipuk et al., 2008). Therefore, from the present study it can be concluded that Moringa peregrina seeds oil showed anti-Leukopenia and antioxidant potential in benzene and/or 5-flurouracil induced Leukopenia, which can be attributed to its flavonoids content. This could serve as a steppingstone towards the discovery of newer safe and effective antitumor agents.

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