WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 115-135 Research Article ISSN 2277– 7105

PROTECTIVE ROLE OF *MORINGA OLEIFERA* LEAF EXTRACT AS A NATURAL ANTIOXIDANT AGAINST TOXIC EFFECT OF PARACETAMOL ON MALE MICE

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Article Received on
11 February 2018,
Revised on 03 March 2018,
Accepted on 25 March 2018,
DOI: 10.20959/wjpr20187-11581

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ABSTRACT

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. It belongs to Moringaceae family. *Moringa oleifera* has been reported to possess anti-cancer, anti-inflammatory and antioxidant effect. The present study was conducted to assess the biochemical and cytogenotoxic effects induced by antipyretic drug Paracetamol on both somatic cells and sperms of male mice and determination of the possible protective role of *Moringa oleifera* as natural antioxidant. Adult male mice of fifteen groups (fifteen animals each) were used in this study: negative control; saline group. Groups 3-6 received Paracetamol at single dose 500, 7 consecutive doses of 500 for seven days, single dose 2000 and7 consecutive doses of 2000 mg / kg for seven days, group 7 received 200 mg/kg. of Moringa for ten consecutive days, groups 8-11 received Moringa before administration of different doses of paracetamol and 12-15 received moringa after administration of the different doses of paracetaml. Reduced glutathione content in liver tissue, frequency of chromosomal aberrations in bone marrow cells and sperm abnormalities were analyzed. The results showed that the paracetamol treatment caused depletion in reduced glutathione content, significant induction of chromosomal aberrations and sperm abnormalities Oral administration of *moringa oleifera* leaf extract either before or after paracetamol treatment was effective in improving the content of hepatic reduced glutathione content (GSH); *moringa oleifera* leaf extract was effective in the reduction of chromosomal aberrations of bone marrow cells and total morphological sperm abnormalities.

KEYWORDS: *Moringa oleifera* leaf extract; Paracetamol; Reduced glutathione; Chromosomal aberrations & Sperm abnormality.

INTRODUCTION

Paracetamol, also known as acetaminophen, N-acetyl-p-aminophenol, 4-hydroxyacetaanilide, PAR or APAP is an effective and widely used antipyretic- analgesic drug with excellent safety record when taken at therapeutic doses.[1,2,3] Paracetamol used for the treatment of pain or fever as part of the flu-like syndrome induced by vaccines or immuno-modulators.[4,5] Paracetamol marketed under many different name brands (most notably Tylenol), and also sold in combination with other drugs, including narcotic compounds such as codeine (Percocet). Paracetamol is primarily metabolized in the liver by sulfation and glucuronidation producing active two-electron oxidation product, N-acetyl-p-benzoquinone imine (NAPQI), by several P450 forms of the microsomal mixed-function oxidase system.[6] In humans and rodents, cytochrome P4502E1 (CYP2E1) and CYP1A2 are the major enzymes of Paracetamol bioactivation, although other P450 forms also oxidize PAR and the relative importance of different forms probably varies among species.[7,8] NAPQI generated from a therapeutic dose of PAR is normally detoxified by the reaction with reduced glutathione (GSH).[9] GSH-derived metabolites are excreted in the urine as mercapturic acid and cysteine conjugates.[10] In overdose, glucuronidation and sulfation pathways become saturated; NAPQI causes GSH depletion, oxidative stress, and binds covalently to liver proteins[9,11] thus paracetamol is selected as hepatotoxicant in inducing injury to the liver at high doses and is still associated with several hundred deaths a year in the United States [12] as it is known to cause hepatotoxicity in human and experimental animals.[2,3] Also [13] observed that paracetamol has genotoxic effect in the mice.

Moringa *oleifera* Lam. is a tree that grows widely in many tropical and subtropical countries. It belongs to Moringaceae.[14,15,16] Moringa *oleifera* has been reported to possess anti-cancer[16] anti-inflammatory[17] antioxidant[18]as the leaves are reported to contain various types of antioxidant compounds such as flavonoids, phenolics and carotenoids;[19,20] also the leaves are excellent source of Vitamin B, Calcium, Protein and Potassium. Beta-carotene and other phytochemicals with known powerful antioxidant ability Kaempferol, Quercetin, Rutin and Caffeoylquinic acids; powerful antioxidant vitamins - C, E, and A and essential micronutrients with antioxidant activity - Selenium and Zinc **Fuglie, (2001); Jaiswal *et al.*, (2009) and Vongsak *et al.*, (2013)**.[21,22,23]

Sathya et al.,2010[24] reported that the pre–treatment with the ethanolic extracts of *M. oleifera* leaves significantly decreased The high percentages of micronuclei and DNA damage induced by cyclophosphamide in mice. Moreover *M. oleifera* leave aqueous extract had a therapeutic action against radiation through increasing of liver enzyme activities and reduction of micronuclei and DNA damage in irradiated rats by gamma irradiation (**Eshak and Osman, 2013**).[25] Also **Radwan et al., (2015)**[26] showed a protective effects of Moringa against genotoxicity of CCL_4.

This study was aimed to evaluate the protective role of ethanolic extracts of *M. oleifera* leaves against the biochemical and cytogenotoxic effects induced by Paracetamol on both somatic cells and sperms of male mice.

MATERIALS AND METHODS

Paracatamol (Acetamenophen)

Paracetamol (Acetamenophen), manufactured by El Nasr Pharmaceutical Chemicals Co. "ADWIC" Abu-Zaabal- A.R.E., dissolved in saline solution (0.9 % Na Cl) and administrated to mice by oral gavage with volume 0.2 ml within two doses 500 and 2000 mg/kg.bwt. either as a single dose or seven consecutive doses within 24 hour interval.

Moringa oleifera

Moringa oleifera which produced by the agriculture division in National Research Centre, Dokki, Egypt.was applied to the animal by oral gavage with volume 0.5 ml at 200mg/kg.bwt. for ten consecutive days either before or after Paracetamol treatments.

Experimental animal

225 Adult male mice (10-12 week-old, weighting 20-25 g) of Swiss strain were obtained from the stock colony of National Research Centre, Dokki, Egypt. Mice were held in polypropylene cages and were housed in a controlled atmosphere with temperature range of 25±5 C˚ and mean relative humidity of 50± 5%. The animals were maintained on commercial mouse pellets *ad libitium* and had free access to water throughout the studies. All animals were cared for and experiments were carried out in accordance with the guidelines for the use of experimental animals and approved by the Ethics Committee of the National Research Centre, Egypt.

Experimental design

Male mice were divided into 15 groups (15 animals/ group) as follow:

Group no.	Description
1	Un treated control.
2	Saline group (0.9 % Na Cl).
3	Oral administration with paracetamol (500 mg/kg b. wt.) as a single dose (SLD).
4	Oral administration with paracetamol (500 mg/kg b. wt.) as a seven consecutive doses with in 24 h. interval (7LD).
5	Oral administration with paracetamol (2000 mg/kg b. wt.) as a single dose SHD.
6	Oral administration with paracetamol (2000 mg/kg b. wt.) as a seven consecutive doses (7HD) with in 24 h. interval.
7	Oral administration with *moringa oleifera* leaf extract (200 mg/kg b. wt.) as ten consecutive doses with in 24 h. interval.
8	Oral administration with *moringa oleifera* leaf extract before a single dose of paracetamol (500 mg/kg) treatment.
9	Oral administration with *moringa oleifera* leaf extract before seven doses of paracetamol (500 mg/kg) treatment.
10	Oral administration with *moringa oleifera* leaf extract before a single dose of paracetamol (2000 mg/kg) treatment.
11	Oral administration with *moringa oleifera* leaf extract before seven doses of paracetamol (2000 mg/kg) treatment.
12	Oral administration with paracetamol (500 mg/ kg) as a single dose before moringa.
13	Oral administration with paracetamol (500 mg/kg b. wt.) as a seven consecutive doses before moringa.
14	Oral administration with paracetamol (2000 mg/ kg) as a single dose before moringa.
15	Oral administration with paracetamol (2000 mg/kg b. wt.) as a seven consecutive doses before moringa.

At the end of the experimentation period, at least one half and hour after treatment with cochicine before collecting bone marrow of femurs of 5 male mice for chromosomal aberrations test. Liver tissue of other 5 animal of each treated group stored at – 80℃ until use for determination of reduced glutathione content (GSH).5 animals from each group were used for the determination of sperm abnormality test and sacrificed after 35 day from the beginning of the treatment; two cauda epididymis were removed for sperm abnormality study.

Determination of reduced glutathione (GSH)

Reduced glutathione content of liver tissue homogenate was measured according to the method described by [27,28]with some modifications. The assay based on the reduction of 5, 5 – dithiobis – (2 – nitrobenzoic acid) (DTNB) by SH groups of glutathione to form 2 – nitro-S-mercaptobenzoic acid per mole of glutathione. The product is measured spectrophotometrically at 412 nm using the extinction coefficient of 13.7 mM^{-1} cm^{-1}.

Studying chromosomal aberrations in bone marrow

The chromosomes were prepared for microscopic examination according to.[29] 50 well spread metaphases were examined for each animal. Both structural (deletion, break, gap and fragment) and numerical aberrations (polyploidy and aneuploidy) were recorded.

Sperm abnormality test

Morphological sperm abnormalities including (head and tail), the cauda epididymidis was removed from each side and minced in 2 ml of saline solution (0.9% NaCl) and processed according to the method described by.[30]

Statistical analysis

The SPSS computer program version 16.0 was used. The statistical analysis was carried out by one- way ANOVA setting the probability level to $p < 0.05$, followed by Waller- Duncan k – ratio. The treated groups were compared both with each other and with either saline group or un treated control group.

RESULTS

Determination of reduced glutathione (GSH)

Table (1) shows the protective effect of Moringa oleifera on GSH content in liver tissue homogenate against depletion induced by Paracetamol at both doses in mice after 24 hr. As summarized in **Table (1);** treatment with Moringa oleifera alone revealed a significant increase in liver GSH level after 24 hr. **(7.91 ± 0.52)** when compared with normal control values measured GSH content after 24 hr. as follow **(5.11 ± 0.19)**. Treatment with Moringa before Paracetamol (500 mg/kg b. wt.) showing a high significant increase in GSH content in liver of mice after 24 hr. as follow **(6.31 ± 0.58**) in comparing to control group **(5.11 ± 0.19)**.Also there is a significant increase in GSH content detected in groups treated with Moringa before Paracetamol at 7 doses of 500 mg, SHD and 7HD compared to control group as follow (**6.5 ± 0.57, 6.06 ± 0.27, 5.71 ± 0.33 and 5.11 ± 0.19** respectively).While there is no significance detected in group treated with *Moringa oleifera* after Paracetamol (500 mg/kg b. wt.) as showed in **Table (2)** as follow **(5.16 ± 0.31**) in comparing to control group as reached to the same manner of the control values. While in groups treated with *Moringa oleifera* after Paracetamol at 7LD, SHD and 7 HD there is no significant detected in GSH content comparing to 7 LD, SHD and 7 HD respectively as showed in **Table (2)** as follow (3.15 ± 0.30, 3.04 ±0.28, 1.76 ± 0.11, 2.83 ± 0.21, 2.63 ± 0.14 & 1.50 ± 0.19 respectively).

Studying chromosomal aberrations in bone marrow

Tables (3 & 4) summarize the protective effect of Moringa oleifera leaf extract either before or after treatment with Paracetamol at all doses (SLD, 7LD, SHD and 7 HD) on total structural aberrations of chromosomes in bone marrow cells. Treatment with *Moringa oleifera* before Paracetamol at all doses (SLD, 7LD, SHD and 7 HD) showing a high significant decrease in total structural aberrations of chromosomes as follow (**24.8 ± 1.43, 33.4 ± 1.63, 40.6 ± 2.73** and **51.8 ± 1.24** respectively) in comparing to Paracetamol treated groups at all doses (**30.4 ± 3.66, 48.2 ± 3.07, 57.8 ± 3.58** and **66.6 ± 5.44** respectively) as showed in **Table (3)**. Whereas, **Table (4)**.showed treatment with *Moringa oleifera* after Paracetamol at a single dose of 500 mg/kg (**28.2 ± 1.11**) showing no significant compared to single low dose of Paracetamol as follow (**30.4 ± 3.66**) Post treatment with *Moringa oleifera* with Paracetamol at the following doses 7LD, SHD and 7 HD as follow (**36.8 ± 2.65, 44.4 ± 1.12** and **48.4 ± 3.57** respectively) illustrated a significant decrease in total structural aberrations of bone marrow chromosomes compared to Paracetamol as showed in **Fig (a, b, c, d, e, f &h)** at the following doses 7LD, SHD and 7 HD as follow (**48.2 ± 3.07, 57.8 ± 3.58** and **66.6 ± 5.44** respectively).

Table (1): Effects of *Moringa oleifera* as a pre-treatment on liver glutathione depletion induced by Paracetamol.

Treatments	Glutathione content in the liver tissues µmol/g tissue	
	M ± SE	Change
Control	5.11 ± 0.19 [b]	0
Solvent	4.72± 0.19[b]	- 0.39
Para.(single dose of 500 mg/kg)	3.44 ± 0.24[c]	- 1.67
Para. (7 doses of 500mg/kg)	2.83 ± 0.21[d]	- 2.28
Para.(single dose of 2000 mg/kg)	2.63 ± 0.14[d]	- 2.48
Para.(7 dose of 2000 mg/kg)	1.50 ± 0.19[e]	- 3.61
Moringa(200mg/kg)	7.91 ± 0.52[a]	+ 2.8
Moringa then Para.(single dose of 500 mg/kg)	6.31± 0.58[a]	+ 1.2
Moringa then Para. (7 doses of 500mg/kg)	6.54 ± 0.57[a]	+ 1.43
Moringa then Para.(single dose of 2000 mg/kg)	6.06 ± 0.27[a]	+ 0.95
Moringa then Para.(7 dose of 2000 mg/kg)	5.71 ± 0.33[b]	+ 0.6

Means with different letters (a, b, c, d and e) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.

Table (2): Effects of *Moringa oleifera* as a post treatment on liver glutathione depletion induced by Paracetamol.

Treatments	Glutathione content in the liver tissues µmol/g tissue

	M ± SE	Change
Control	5.11 ± 0.19 ^{b}	0
Solvent	4.72± 0.19^{b}	- 0.39
Para.(single dose of 500 mg/kg)	3.44 ± 0.24^{c}	- 1.67
Para. (7 doses of 500mg/kg)	2.83 ± 0.21^{c}	- 2.28
Para.(single dose of 2000 mg/kg)	2.63 ± 0.14^{c}	- 2.48
Para.(7 dose of 2000 mg/kg)	1.50 ± 0.19^{d}	- 3.61
Moringa(200mg/kg)	7.91 ± 0.52^{a}	+ 2.8
Para.(single dose of 500 mg/kg) then Moringa	5.16 ± 0.31^{b}	+ 0.05
Para. (7 doses of 500mg/kg) then Moringa	3.15 ± 0.30^{c}	-1.96
Para.(single dose of 2000 mg/kg) then Moringa	3.04 ± 0.28^{c}	- 2.07
Para.(7 dose of 2000 mg/kg) then Moringa	1.76 ± 0.11^{d}	- 3.35

Means with different letters (a, b, c and d) between groups in the same column are significantly different at P<0.05. Animals in each group n= 5. Abbrevations: Para. means Paracetamol.

Table (3): Effects of *Moringa oleifera* as a pre-treatment on structural aberrations of chromosomes induced by Paracetamol.

Treatment	deletion	fragment	PCA	TCA	Total Structural aberration of chromosomes
Control	5.4± 0.4^{g}	0.4±0.24^{c}	8.6±2.68^{d}	0.4±0.4^{d}	14.8±1.74^{e}
Solvent	4.0± 1.14^{g}	0.2±0.2^{c}	7.4±2.41^{d}	3.0±0.89^{c}	14.6±2.11^{e}
Para.(single dose of 500 mg/kg)	17.6±3.84^{c}	0.4±0.4^{c}	11.8±1.41^{c}	0.6±0.4^{d}	30.4±3.66^{cd}
Para. (7 doses of 500mg/kg)	24.8±3.89^{b}	2.0±0.45^{a}	11.4±1.42^{c}	10.0±1.34^{a}	48.2±3.07^{b}
Para. (single dose of 2000 mg/kg)	42.4±1.99^{a}	1.0±0.32^{ab}	10.2±2.30^{c}	4.2±0.49^{b}	57.8±3.58^{a}
Para. (7 dose of 2000 mg/kg)	31.0±6.06^{ab}	2.0±0.95^{a}	23.8±3.13^{b}	9.8±2.18^{a}	66.6±5.44^{a}
Moringa (200mg/kg)	3.0±1.14^{g}	0±0^{c}	7.4±1.56^{d}	0.6±0.24^{d}	11.0±1.82^{e}
Moringa then Para. (single dose of 500 mg/kg)	10.0±0.95^{e}	0.80±0.49^{b}	11.8±2.44^{c}	2.2±0.37^{c}	24.8±1.43^{d}
Moringa then Para. (7 doses of 500mg/kg)	8.8±1.07^{ef}	0.2±0.2^{c}	21.6±2.46^{b}	2.8±0.2^{c}	33.4±1.63^{c}
Moringa then Para. (single dose of 2000 mg/kg)	17.0±4.51^{c}	0.6±0.4^{b}	20.8±3.61^{b}	2.2±0.86^{c}	40.6±2.73^{b}
Moringa then Para. (7 dose of 2000 mg/kg)	11.8±1.07^{d}	0.4±0.4^{c}	35.8±0.88^{a}	3.8±0.86^{b}	51.8±1.24^{b}

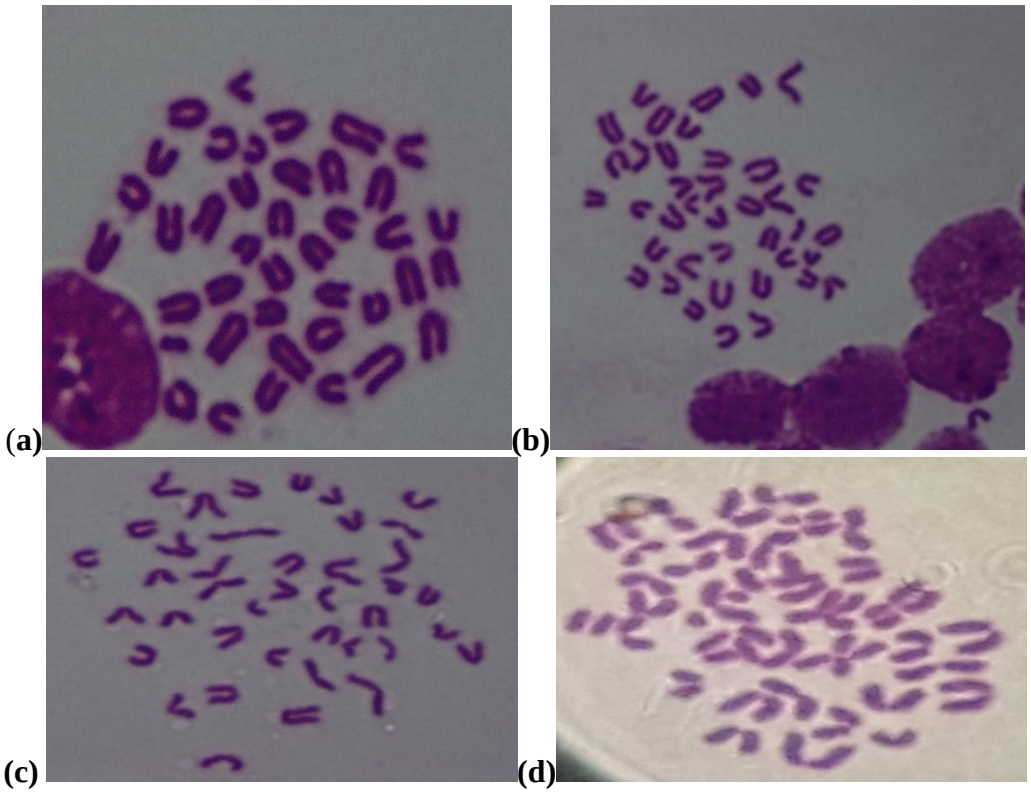
Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.

Table (4): Effects of *Moringa oleifera* as a post-treatment on structural aberrations of chromosomes induced by Paracetamol.

Treatment	deletion	fragment	PCA	TCA	Total Structural

					aberration of chromosomes
Control	5.4± 0.4^{f}	0.4±0.24^{b}	8.6±2.68^{e}	0.4±0.4^{e}	14.8±1.74^{de}
Solvent	4.0± 1.14^{f}	0.2±0.2^{bc}	7.4±2.41^{e}	3.0±0.89^{c}	14.6±2.11^{e}
Para. (single dose of 500 mg/kg)	17.6±3.84^{c}	0.4±0.4^{b}	11.8±1.41^{d}	0.6±0.4^{e}	30.4±3.66^{c}
Para. (7 doses of 500mg/kg)	24.8±3.89^{b}	2.0±0.45^{a}	11.4±1.42^{d}	10.0±1.34^{a}	48.2±3.07^{b}
Para. (single dose of 2000 mg/kg)	42.4±1.99^{a}	1.0±0.32^{a}	10.2±2.30^{d}	4.2±0.49^{b}	57.8±3.58^{a}
Para. (7 dose of 2000 mg/kg)	31.0±6.06^{ab}	2.0±0.95^{a}	23.8±3.13^{b}	9.8±2.18^{a}	66.6±5.44^{a}
Moringa (200mg/kg)	3.0±1.14^{f}	0±0^{c}	7.4±1.56^{e}	0.6±0.24^{de}	11.0±1.82^{e}
Para. (single dose of 500 mg/kg) then Moringa	7.8±1.71^{e}	0.4±0.4^{b}	18.2±1.30^{c}	1.8±0.73^{d}	28.2±1.11^{cd}
Para. (7 doses of 500mg/kg) then Moringa	9.4±1.03^{d}	0±0^{c}	23.6±2.72^{b}	3.8±1.56^{c}	36.8±2.65^{c}
Para. (single dose of 2000 mg/kg) then Moringa	10.4±2.01^{d}	0±0^{c}	31.0±3.06^{a}	3.0±1.22^{c}	44.4±1.12^{b}
Para. (7 dose of 2000 mg/kg) then Moringa	14.4±3.26^{c}	0±0^{c}	28.8±2.68^{a}	5.2±1.46^{b}	48.4±3.57^{b}

Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.



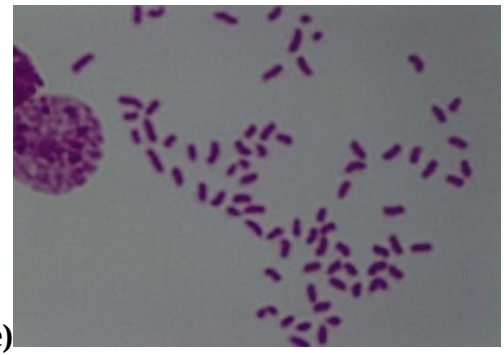
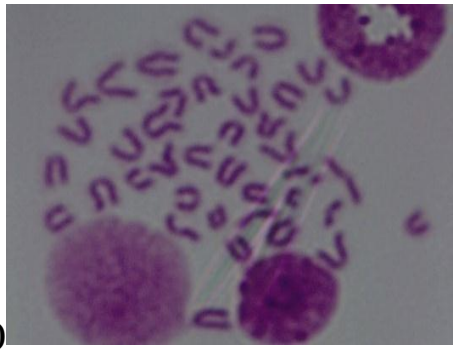
(e)  (f) 

Fig (a) Normal bone marrow metaphase chromosomes in both structure and number.

(b) Bone marrow chromosomes in metaphase with deletion in structure.

(c) Bone marrow chromosomes in metaphase with deletion structure.

(d) Bone marrow chromosomes in metaphase with partial centromeric attenuation in structure.

(e) Bone marrow chromosomes in metaphase with total centromeric attenuation in structure.

(f) Bone marrow chromosomes in metaphase with fragment in structure.

Tables (5 &6) illustrates the protective effect of *Moringa oleifera* leaf extract administrated orally to mice either before or after Paracetamol; which induce numerical aberrations of chromosomes in bone marrow cells of male mice after 24 h. from last treatment as follow SLD, 7 LD, SHD and 7 HD showed a significant *(P < 0.05)* increase (**13.8 ± 1.46, 18.4 ± 0.68, 17.8 ± 0.86 & 24.0 ± 1.38** respectively) compared to control group (**8.6 ± 1.69**). Whereas, animals treated with *Moringa oleifera* alone showed insignificant change on total numerical aberrations of bone marrow chromosomes (**7.2 ± 0.37**) in compared with control group (**8.6 ± 1.69**) as showed in **Tables (5 & 6)**.There is no significant detected in a group of mice treated with Moringa before 500 mg/kg of Paracetamol (**10.4 ± 1.47**) compared to control group (**8.6 ± 1.69**). Whereas, there is a slight significance detected on groups of mice pretreated with *Moringa oleifera* and Paracetamol 7LD, SHD and 7 HD as follow (**15.4 ± 0.98, 14.2 ± 1.66 and 18.2 ± 1.32** respectively) compared to control group (**8.6 ± 1.69**) as showed in **Table (5)**.Using *Moringa oleifera* as a post treatment with mice treated with 7 dose of 500 mg/kg, single dose of 2000 mg/kg and 7 dose of 2000mg/kg showed a significant increase on total numerical aberrations of bone marrow chromosomes (**14.2 ± 2.06, 16.2 ± 1.11 & 19.8 ± 0.86** respectively) compared to control group (**8.6 ± 1.69**) as illustrated in **Table (6)**.

Table (5): Effect of *Moringa oleifera* as a pre-treatment on numerical aberrations of bone marrow chromosomes induced by Paracetamol.

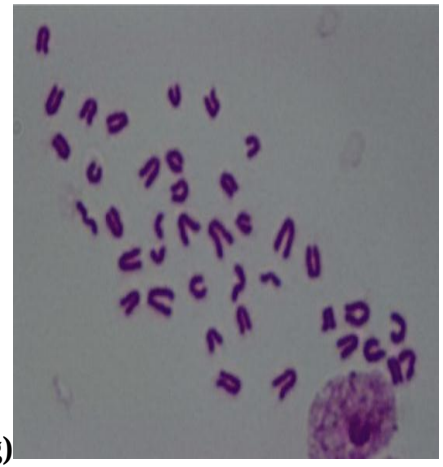
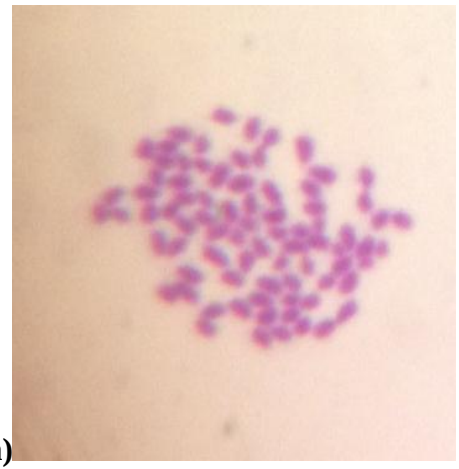
Treatment	Endomitosis	polyploidy	Hypo(-2)	Hypo(-1)	Hyper+1	Hyper+2	Total numerical aberration.
Control	0.4± 0.4^{c}	0.2±0.2^{cd}	3.6±1.03^{c}	3.2±0.92^{d}	1.2±0.58^{c}	0±0^{d}	8.6±1.69^{c}
Solvent	3.6±0.75^{b}	1.0±0.45^{c}	1.0±0.55^{c}	0.8±0.58^{d}	2.2±0.73^{b}	0.2±0.2^{c}	8.8±0.49^{c}
Para. (single dose of 500 mg/kg)	1.0±0.45^{b}	1.4±0.24^{b}	5.4±1.36^{a}	4.4±1.17^{bc}	1.2±0.58^{c}	0.4±0.24^{c}	13.8±1.46^{b}
Para. (7 doses of 500mg/kg)	7.4±1.12^{a}	0.4±0.24^{c}	4.0±0.71^{b}	3.8±0.8^{c}	1.2±0.58^{c}	1.6±0.24^{ab}	18.4±0.68^{a}
Para.(single dose of 2000 mg/kg)	5.6±1.72^{a}	0.6±0.4^{c}	4.0±0.84^{b}	6.6±1.29^{a}	1.0±0.32^{c}	0±0^{d}	17.8±0.86^{b}
Para. (7 dose of 2000 mg/kg)	3.6±0.87^{ab}	1.4±0.6^{b}	4.2±1.24^{a}	9.2±1.62^{a}	3.4±0.93^{a}	2.2±0.58^{a}	24.0±1.38^{a}
Moringa(200mg/kg)	0.6±0.4^{c}	0.8±0.2^{c}	2.4±0.6^{c}	3.2±0.86^{d}	0.2±0.2^{d}	0±0^{d}	7.2±0.37^{c}
Moringa+ Para. (single dose of 500 mg/kg)	0.4±0.24^{c}	0.8±0.49^{c}	2.6±1.21^{c}	5.8±0.8^{b}	0.8±0.58^{cd}	0±0^{d}	10.4±1.47^{c}
Moringa+ Para. (7 doses of 500mg/kg)	2.2±0.8^{b}	3.0±0.95^{a}	3.4±0.4^{c}	4.6±0.68^{b}	1.8±0.37^{b}	0.4±0.4^{c}	15.4±0.98^{b}
Moringa + Para. (single dose of 2000 mg/kg)	1.4±0.4^{b}	2.0±0.63^{a}	4.8±1.53^{a}	2.8±0.66^{d}	1.6±0.4^{bc}	1.6±0.51^{ab}	14.2±1.66^{b}
Moringa + Para. (7 dose of 2000 mg/kg)	1.4±0.6^{b}	2.0±0.63^{a}	4.0±0.84^{b}	6.4±0.51^{b}	2.8±0.92^{b}	1.6±0.51^{ab}	18.2±1.32^{ab}

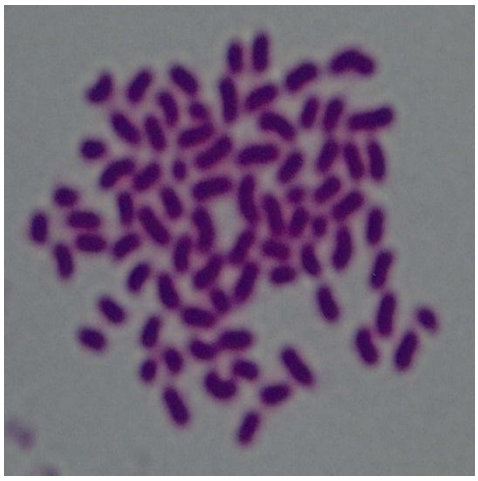
Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.

Table (6): Effects of *Moringa oleifera* as a post-treatment on numerical aberrations of bone marrow chromosomes induced by Paracetamol.

Treatment	Endomitosis.	Polyploidy	Hypo (-2)	Hypo (-1)	Hyper (+1)	Hyper (+2)	Total numerical aberrations
Control	0.4± 0.4^{d}	0.2±0.2^{c}	3.6±1.03^{c}	3.2±0.92^{c}	1.2±0.58^{c}	0±0^{b}	8.6±1.69^{d}
Solvent	3.6±0.75^{b}	1.0±0.45^{b}	1.0±0.55^{c}	0.8±0.58^{c}	2.2±0.73^{b}	0.2±0.2^{b}	8.8±0.49^{d}
Para.(single dose of 500 mg/kg)	1.0±0.45^{c}	1.4±0.24^{b}	5.4±1.36^{b}	4.4±1.17^{b}	1.2±0.58^{c}	0.4±0.24^{b}	13.8±1.46^{c}
Para. (7 doses of 500mg/kg)	7.4±1.12^{a}	0.4±0.24^{c}	4.0±0.71^{b}	3.8±0.8^{b}	1.2±0.58^{c}	1.6±0.24^{a}	18.4±0.68^{b}
Para.(single dose of 2000 mg/kg)	5.6±1.72^{a}	0.6±0.4^{c}	4.0±0.84^{b}	6.6±1.29^{ab}	1.0±0.32^{c}	0±0^{b}	17.8±0.86^{b}
Para.(7 dose of 2000 mg/kg)	3.6±0.87^{b}	1.4±0.6^{ab}	4.2±1.24^{b}	9.2±1.62^{a}	3.4±0.93^{a}	2.2±0.58^{a}	24.0±1.38^{a}
Moringa(200mg/kg)	0.6±0.4^{d}	0.8±0.2^{c}	2.4±0.6^{c}	3.2±0.86^{c}	0.2±0.2^{d}	0±0^{b}	7.2±0.37^{d}
Para.(single dose of 500 mg/kg) + Moringa	2.2±0.58^{b}	1.4±0.68^{a}	3.0±0.95^{c}	4.4±1.12^{b}	0.6±0.24^{d}	0±0^{b}	11.6±1.21^{d}
Para. (7 doses of 500mg/kg) + Moringa	0±0^{d}	1.0±0.63^{b}	7.8±1.62^{a}	4.2±0.58^{b}	1.0±0.55^{c}	0.2±0.2^{b}	14.2±2.06^{c}
Para.(single dose of 2000 mg/kg) + Moringa	0.2±0.2^{d}	0.6±0.6^{c}	7.0±0.55^{a}	7.0±1.1^{a}	0.8±0.37^{c}	0.6±0.4^{b}	16.2±1.11^{c}
Para.(7 dose of 2000 mg/kg) + Moringa	0.4±0.4^{d}	1.6±0.81^{a}	7.4±1.17^{a}	9.2±1.59^{a}	1.0±0.63^{c}	0.2±0.2^{b}	19.8±0.86^{b}

Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol

(g)  **(h)** 

(i) 

(g) Normal bone marrow metaphase chromosomes in structure while it is hypoploidy (-1). (h) Endomitosis. (I) Polyploidy.

Morphological sperm abnormality test

Tables (7 & 8) illustrated the protective effect of *Moringa oleifera* in reducing the total morphological sperm abnormalities. Treatment with Paracetamol at both doses (500 & 2000 mg/kg) either as a single dose or 7 consecutive doses as follow (**44.8 ± 2.29, 55.4 ± 2.29, 54.8 ± 3.34 & 83.6 ± 4.99** respectively) induce the increase in total morphological sperm abnormalities as showed in **Table (7)** compared to control group (**13.2 ± 2.27**). Also as illustrated in **Table (7)** treatment with *Moringa oleifera* alone showed a significant decrease in total morphological sperm abnormalities (**11.6 ± 0.87**) comparing to control group (**13.2 ± 2.27**).Treatment with *Moringa oleifera* before Paracetamol (SLD, 7 LD, SHD & 7 HD) showing a high significant reduction in total morphological sperm abnormalities (22.8± 0.66, 32.4 ± 0.87, 35.0 ± 1.1 & 48.0 ± 2.1 respectively) comparing to groups treated with Paracetamol (SLD, 7 LD, SHD & 7HD) as follow (**44.8 ± 2.29, 55.4 ± 2.29, 54.8 ± 3.34 & 83.6 ± 4.99** respectively).While treatment with *Moringa oleifera* after Paracetamol treatment

(SLD, 7 LD, SHD & 7 HD) showing a slight significance in reduction of total morphological abnormalities of sperm as follow (22.2 ± 1.28, 44.0 ± 2.17, 43.6 ± 2.11 & 53.6 ± 0.81 respectively) compared to Paracetamol at both doses (500 & 2000 mg/kg) either as a single dose or 7 consecutive doses as follow (**44.8 ± 2.29, 55.4 ± 2.29, 54.8 ± 3.34 & 83.6 ± 4.99** respectively)so treatment with Moringa oleifera before Paracetamol is better than after Paracetamol.

The abnormality in sperm morphology of Paracetamol treated groups (at 500 and 2000 single therapeutic doses) showed a significant ($P<0.05$) increase **(44.8 ± 2.29** and **54.8 ± 3.34** respectively) compared to control group **(13.2 ± 2.27)**. Whereas, animals treated with Moringa oleifera alone showed insignificant changed in sperm morphology **(11.6 ± 0.87**) in compared with the control group **(13.2 ± 2.27)** as illustrated in **Tables (7 &8)**. There are different morphological abnormalities in sperm in both head and tail as a sperm with amorphous head that taken different shapes as illustrated in **Fig (j)**, the head without hook as showed in **Fig (k),** the banana and normal of head shape as showed **in Fig (l),** also abnormalities in tail as coiling as showed in **Fig (m)**.

Table (7): Effects of *Moringa oleifera* as a pre-treatment on sperm abnormalities induced by Paracetamol.

Treatments	Sperm tail abnormalities	Sperm head abnormalities			Total sperm abnormalities / 1000 sperm	
	Coiled	Amorphous	Without-hock	Banana	Mean ± SE	Range
Control	0.60 ± 0.25^{d}	7.0 ± 1.87^{d}	4.20 ± 1.80^{e}	1.4 ± 0.25^{b}	13.20 ± 2.27^{f}	8 – 19
Solvent	0.60 ± 0.40^{d}	13.20 ± 1.59^{c}	2.8 ± 0.86^{e}	1.4 ± 0.25^{b}	18.0 ± 2.20^{f}	11 – 23
Para.(single low dose)	3.0 ± 1.01^{c}	24.0 ± 1.87^{a}	16.80 ± 1.02^{c}	1.0 ± 0.45^{b}	44.8 ± 2.29^{c}	39 – 52
Para. (7 low doses)	4.0 ± 0.63^{c}	20.40 ± 3.46^{b}	28.60 ± 2.36^{b}	2.4 ± 0.25^{a}	55.40 ± 2.29^{b}	51 – 64
Para.(single high dose)	9.8 ± 2.60^{a}	18.40 ± 2.11^{b}	24.20 ± 2.42^{b}	2.2 ± 0.37^{a}	54.80 ± 3.34^{b}	46 – 63
Para.(7 high doses)	12.20 ± 1.39^{a}	26.40 ± 3.17^{a}	44.00 ± 6.27^{a}	1.0 ± 0.78^{b}	83.60 ± 4.99^{a}	72 – 98
Moringa(200mg/kg)	0.60 ± 0.25^{d}	4.60 ± 1.25^{d}	4.80 ± 1.53^{e}	1.4 ± 0.51^{b}	11.60 ± 0.87^{f}	9 – 14
Moringa+ single low dose	3.80 ± 2.11^{c}	9.0 ± 1.14^{c}	9.80 ± 1.56^{d}	0.2 ± 0.20^{c}	22.80 ± 0.66^{e}	21 - 25
Moringa+ 7 low doses	10.20 ± 2.10^{a}	12.60 ± 2.06^{c}	9.40 ± 1.25^{d}	0.2 ± 0.2^{c}	32.40 ± 0.87^{d}	30 - 35
Moringa+Single high dose	9.40 ± 3.19^{b}	18.80 ± 2.22^{b}	6.20 ± 1.24^{d}	0.6 ± 0.4^{e}	35.0 ± 1.10^{d}	32 - 38
Moringa + 7 high doses	7.60 ± 1.94^{b}	21.00 ± 3.15^{b}	18.20 ± 3.28^{c}	1.2 ± 0.58^{b}	48.0 ± 2.10^{b}	41 - 54

Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.

Table (8): Effects of *Moringa oleifera* as a post-treatment on sperm abnormalities induced by Paracetamol.

Treatments	Sperm tail abnormalities	Sperm head abnormalities			Total sperm abnormalities / 1000 sperm	
	Coiled	Amorphous	Without-hock	Banana	Mean ± SE	Range
Control	0.60 ± 0.25^{d}	7.0 ± 1.87^{c}	4.20 ± 1.80^{e}	1.4 ± 0.25^{b}	13.20 ± 2.27^{e}	8 – 19
Solvent	0.60 ± 0.40^{d}	13.20 ± 1.59^{b}	2.8 ± 0.86^{e}	1.4 ± 0.25^{b}	18.0 ± 2.20^{d}	11 – 23
Para.(single low dose)	3.0 ± 1.01^{c}	24.0 ± 1.87^{a}	16.80 ± 1.02^{c}	1.0 ± 0.45^{c}	44.8 ± 2.29^{c}	39 – 52
Para. (7 low doses)	4.0 ± 0.63^{c}	20.40 ± 3.46^{a}	28.60 ± 2.36^{b}	2.4 ± 0.25^{a}	55.40 ± 2.29^{b}	51 – 64
Para.(single high dose)	9.8 ± 2.60^{b}	18.40 ± 2.11^{b}	24.20 ± 2.42^{b}	2.2 ± 0.37^{a}	54.80 ± 3.34^{b}	46 – 63
Para.(7 high doses)	12.20 ± 1.39^{a}	26.40 ± 3.17^{a}	44.00 ± 6.27^{a}	1.0 ± 0.78^{c}	83.60 ± 4.99^{a}	72 – 98
Moringa(200mg/kg)	0.60 ± 0.25^{d}	4.60 ± 1.25^{cd}	4.80 ± 1.53^{e}	1.4 ± 0.51^{b}	11.60 ± 0.87^{e}	9 – 14
Single low dose + Moringa	3.40 ± 0.68^{c}	8.6 ± 0.68^{c}	9.2 ± 1.16^{d}	1.0 ± 0.45^{c}	22.2 ± 1.28^{de}	20 – 26
7 low doses + Moringa	15.60 ± 3.93^{a}	11.6 ± 2.38^{bc}	14.2 ± 1.16^{c}	2.6 ± 0.75^{a}	44.0 ± 2.17^{c}	38 – 49
Single high dose+ Moringa	14.0 ± 5.22^{a}	13.2 ± 3.81^{b}	12.4 ± 3.34^{cd}	0	43.6 ± 2.11^{c}	39 – 50
7 high doses+ Moringa	11.6 ± 4.76^{ab}	25.8 ± 4.25^{a}	16.2 ± 1.24^{c}	0	53.6 ± 0.81^{b}	52 – 56

Means with different letters (a, b, c, d, e, f and g) between groups in the same column are significantly different at P<0.05. Animals in each group (n= 5). Abbrevations: Para. means Paracetamol.

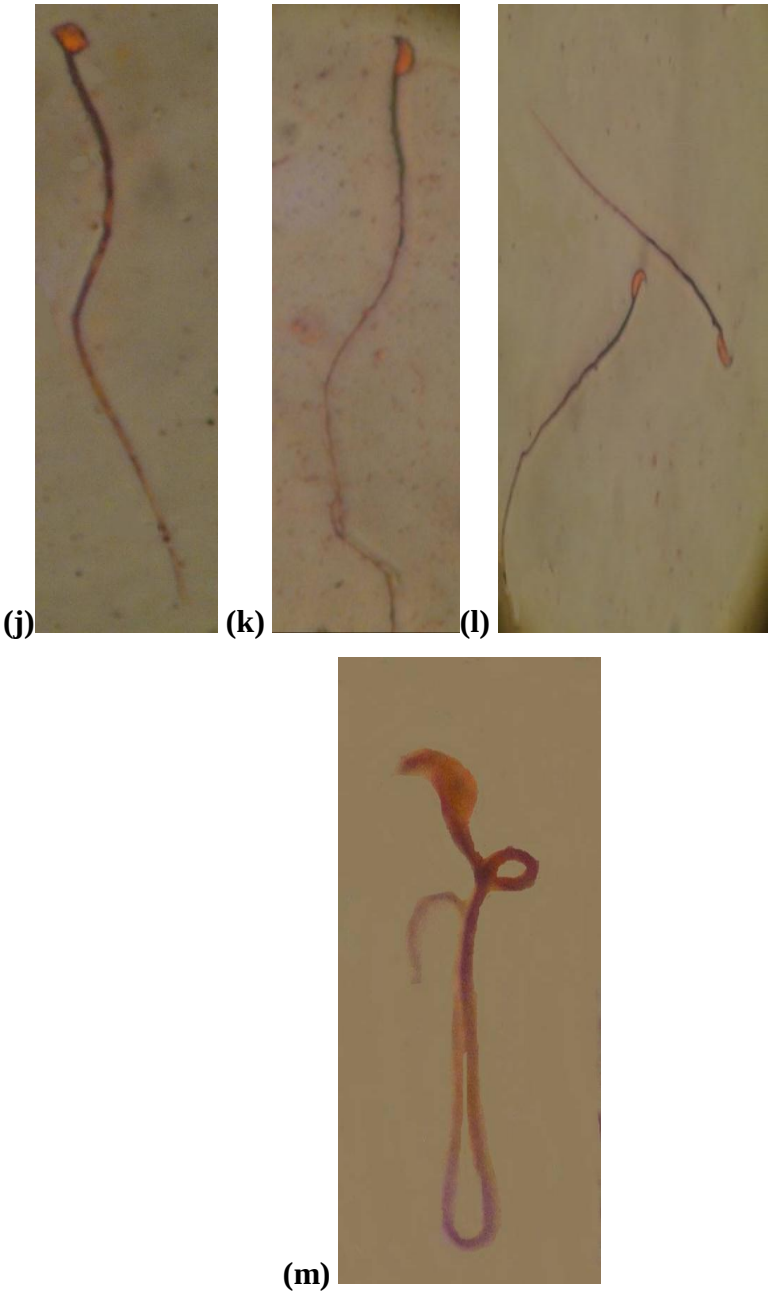


Fig (j) amorphous head, Fig (k) the head without hook, Fig (l) the banana and normal of head shape, Fig(m) abnormalities in tail as coiling.

DISCUSSION

From data of the present work, when GSH was measured in animals treated with paracetamol treatment at both doses either as a single dose or 7 consecutive doses induced a significant ($p<0.05$) depletion in the liver GSH content after 24 h. from last treatment when compared to the control. The depletion of reduced glutathione due to the formation of reactive metabolites is somehow triggers the cascade events of hepatotoxicity.[31] Overdose of paracetamol results in the generation of free radicals following the depletion of glutathione.[31]

The present results illustrated in **Tables (1 & 2)** shows that there is a significant increase in GSH in liver tissue among *moringa oleifera* treated mice and control group. Moreover, the current data illustrated that *moringa oleifera* leaf extract pre- or post-treatment to paracetamol significantly increased the liver GSH content when compared to paracetamol treated groups at both doses (500 & 2000 mg/kg b.wt.) either as a single dose or 7 consecutive doses. These results agreed with the results obtained from [32]showed that the administration of 200 and 800 mg/kg of aqueous ethanol extracts of *Moringa oleifera* leaves prevented acetaminophen-induced liver damage as determined by decreases in AST, ALT, and ALP as well as increases in hepatic glutathione. Also, Our results were in agreement with results induced by [14]and [33]demonstrated that intraperitoneal administration of hydroethanol extracts of *Moringa oleifera* leaf and flower protected against acetaminophen-induced liver damage. Also the oral administration of Moringa oleifera extract to rats resulted in significant improvemen in GSH content in liver cells.[34]

The extracts increased glutathione levels and increased the levels of the antioxidant enzymes superoxide dismutase and catalase. *Moringa oleifera* has been reported to possess antioxidant[18] as the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolic and carotenoids.[13,20]

In the current study, the possible protective effect of *moringa oleifera* as a natural antioxidant against genotoxicity and cytotoxicity induced by paracetamol has been evaluated. Paracetamol is an indirect acting agent as in humans and rodents, cytochrome P4502E1 (CYP2E1) and CYP1A2 are the major enzymes of Paracetamol bioactivation.[7,8]

In the present study, the results of chromosomal aberrations of bone marrow induced after the treatment of animals with paracetamol either as a single dose or 7 consecutive doses of both doses (500 & 2000 mg/kg b.wt.) exhibited a significant increased of chromosomal abnormality in both (structure & number) of bone marrow compared to control group as illustrated in **Tables (3, 4, 5 & 6)**. The present study results agreed with the results induced by [13] that Paracetamol treatment induced highly significant increases of all types of chromosomal aberrations compared to the control.The most frequent aberrations were chromatid deletions, followed by chromatid breaks and fragments. Paracetamol reduces DNA synthesis by a specific inhibition of ribonucleotide reductase, including SCE and CA as breaks and chromatic exchanges.[35]

While there is a significant decreased in total structural aberrations detected in groups of animals treated with *Moringa oleifera* alone comparing to groups treated with Paracetamol as showed in **Tables (3 & 4)**. Also there is a significant decreased in total structural aberrations of bone marrow chromosomes in groups pre-treated with *Moringa oleifera* comparing to groups treated with Paracetamol alone as illustrated in **Table (4)**.While there is a slight decrease in total structural aberrations of bone marrow chromosomes in groups post-treated with *Moringa oleifera* leaf extract as showed in **Table (4)** compared to groups treated with Paracetamol alone. Also from results revealed in **Tables (5 & 6)**, there is a significant increased in total numerical aberrations of bone marrow chromosomes detected in groups treated with Paracetamol either as a single dose or seven consecutive doses of 500 and 2000 mg/kg compared to control group).While there is no significant detected in group treated with *Moringa oleifera* comparing to control group.These results agreed with previous studies that observed The antimutagenic phytoconstituents of Moringa were minimized of cytotoxicity and genotoxicity that induced in mice and rats by exposure of radiation[36,25]and the treatment with cyclophosphamide and CCL_4.[24,26]

Sperm abnormalities in Paracetamol groups might be due to attack of generated ROS to polyunsaturated fatty acid residues of phospholipids of cell membrane occurring lipid peroxidation (LPO). So the sperms are highly sensitive to oxidative stress because they have a high content of polyunsaturated fatty acids in the plasma membrane. Increased LPO and altered membrane can damage the sperm DNA causing sperm abnormalities.[37,38] In the present study, for improving the mutagenic effect of Paracetamol on the structure of sperms, *Moringa oleifera* aqueous leaf extract was administrated to mice orally as ten consecutive doses within 24 hr. interval with a volume of 0.5 ml at 200 mg/ kg either before or after Paracetamol treatment. From results showed in **Tables (7 & 8)** treatment with Moringa oleifera before Paracetamol have a significant reduction in the total morphological sperm abnormalities comparing to Paracetamol treated groups eiher low doses or high does. The present study results proved the results of others before as follow[39] proved that the sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme system levels by Moringa treatment can favour the reproductive process and also enhances spermatogenesis, as Moringa oleifera leaves are excellent source of Vitamin B, Calcium, Protein and Potassium. Beta-carotene and other phytochemicals were known powerful antioxidant ability – Kaempferol, Quercetin, Rutin and Caffeoylquinic acids; powerful antioxidant vitamins - C, E, A and essential micronutrients with antioxidant activity

- Selenium and Zinc as explained by.[23][40] found that methanolic extract of Moringa does not affect sexual behaviour or serum androgen level but enhances seminiferous tubules, testis and Epididymal weight and seminal vesicles in the male rats.

In conclusion, this study revealed that Moringa has protective effect as natural antioxidant against genotoxicity of Paracetamol treatment.

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