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Ameliorative Effect of Moringa Seed Oil Against Chlorambucil-Induced DNA Damage and Hepatotoxicity in Adult Male Albino Rats

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ABSTRACT

Moringa oil was potentially effective therapy in the treatment of xenobiotic-induced liver disorders. The present study was carried out to investigate the biological effects of moringa oil against hepatic injury and DNA damage induced by chlorambucil in rats. Forty male rats were divided into four groups. Group I: rats maintained as control, group II: rats received moringa oil, group III: rats received chlorambucil, group IV: rats received chlorambucil and moringa oil. At the end of the fourth week, data showed that oral administration of chlorambucil significantly increased (p<0.001) both cholesterol and triglycerides levels when compared with the control rats. Histopathological results showed that chlorambucil produced cellular damage and severe inflammatory lesions in the liver compared to the control group. Ultrastructural examination of the liver revealed many changes. Pyknotic nuclei were seen and the majority of mitochondria had excessively dense matrices while rough endoplasmic reticulum showed disorganization and dilation of their cisternae. Cytogenetically, hepatocytes of chlorambucil treated rats revealed significant DNA damage. Administration of moringa oil alongside with chlorambucil significantly reversed chlorambucil-induced toxicity in the hepatic tissue. These data demonstrate that moringa oil exhibits potent hepatoprotective effects on chlorambucil-induced liver damage in rats through the prevention of DNA damage.

Keywords: Chlorambucil, Moringa oil, Hepatotoxicity, Histopathology, Ultrastructure, DNA damage.

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INTRODUCTION

Cancer has long been the gravest challenge to human health, which not only reduces the quality of life but also increases mortality. Cancer is the first or second leading cause of death globally before age 70 years in 91 of 172 countries [1]. Cancer therapies possess several side effects including toxicity to normal cells and drug resistance problems [2].

Chlorambucil (CLB), is the most widely cytotoxic alkylating agent of nitrogen mustards. It is bi-functional alkylating exogenous agent that break DNA by forming guanine nucleotide and creating an intrastrand guanine-guanine crosslink [3]. Chlorambucil created hepatic toxicity in mice through an increase in aminotransferases activities and the appearance of massive necrosis in the adjacent parenchyma with proliferative bile duct epithelium [4]. Chlorambucil increased alkaline phosphatase activity and bilirubin and malondialdehyde levels while it reduced the activities of hepatic catalase, superoxide dismutase and glutathione-S-transferase. It also caused liver architecture damage and periportal cellular infiltration [5].

Throughout human history, many bioactive compounds and plant extracts are used as a therapeutic traditional treatment for different diseases [6]. *Moringa oleifera* (MO) is one of the plants which have high nutritional and medicinal values to many societies. Extracts of MO parts such as seed cotyledon, seed coat, stem bark, leaves and root bark were reported to possess antimicrobial potential [7].

Moringa oleifera has been grown under Mediterranean conditions [8]. Moringa seed crude oil extract mainly contains sterols such as campesterol, stigmasterol, β-sitosterol, Δ5-avenasterol and clerosterol. There were also minute amounts of 24-methylenecholesterol, Δ7-campestanol, stigmastanol and 28-isoavenasterol. It also considered as a particular origin of minor components such as tocopherols (α, γ and δ) [9].

Moringa oleifera showed a strong and significant antioxidant activity [10]. *Moringa oleifera* seed extract can subside liver fibrosis and controlled the rising of serum aminotransferases activities and globulin levels induced by carbon tetrachloride [11]. The hepatoprotective ability of MO seed oil against liver damage induced by acetaminophen was reported by Olatosin *et al*. [12]. Moringa oil was also potentially effective therapy in the treatment of xenobiotic-induced liver disorders [13]. Hydroethanolic extracts of edible parts of moringa significantly alleviated the liver damage through their antioxidant nature [14]. The current work was designed to determine the protective potential and the possible ameliorative role of moringa oil on chlorambucil-induced hepatotoxicity and DNA damage in rats.

MATERIALS AND METHODS

Drug and chemicals

Chlorambucil, known as Leukeran, was obtained in the form of tablets manufactured by the Pharmaceutical Company GlaxoSmithKline, Germany. Each tablet contains 2mg chlorambucil. The tablets were dissolved in distilled water. Assay kits for cholesterol and triglycerides were obtained from Spinreact, S.A. Ctra. Santa Coloma, Spain. Chemicals used for comet assay were obtained from Sigma Chemicals Co. (St. Louis, MO). All chemicals used were of analytical grade obtained from Chemical Kits Companies in Egypt.

Plant materials

Moringa seed oil was purchased from Pure Life Company for Investment and Agricultural Development, Giza, Egypt. The physical and chemical characteristics of moringa oil were recorded by Ruttarattanamongko *et al*. [15].

Animal selection and care

Forty, three-month old, healthy adult male albino rats (*Rattus norvegicus*), approximately 140±3 g, were used for the study. They were procured from Animal House Colony of the National Research Centre, Dokki, Giza, Egypt. Rats were housed in clean plastic cages, fed with rodent pellet diet and water was allowed *ad libitum*. They were acclimatized at controlled temperature (24 ± 2°C), light set at a 12 h light-dark cycle, for 2 weeks before starting the experiment.

The rats were equally randomized into four groups (10 rats in each) and were treated as follows:

Control group (GI): Rats were negative control received distilled water.

Moringa oil group (GII): Rats were orally administered moringa oil by gastric intubation at a dose level of 2 ml/Kg b.w/day for four weeks [12].

Chlorambucil group (GIII): Rats were orally given CLB by gastric intubation at a dose level of 1.26 mg/ Kg b.w/day (equivalent to the therapeutic dose for human) [16], for four weeks.

Chlorambucil and moringa oil group (GIV): Rats were orally administered CLB and then after one h, they were given moringa oil (at exact dosages that received by the rats in group III and II respectively) daily for four weeks.

Effect of the different treatments on body weight of the rats

The body weight of each rat of all the experimental groups was recorded at the beginning and at the end of the experimental period, before the experimental diet administration and at necropsy (post-fasting), to see the effect of the different treatments on the animals.

Samples collection

At the end of the experiment, animals of all groups were kept fasting for 12 h, then sacrificed under ether anesthesia. The blood samples were withdrawn from the heart in plain tubes and left to clot, then centrifuged for 10 min at 3000 rpm to obtain clear sera. Sera were stored at –20°C and then used to determine the levels of cholesterol and triglycerides.

Liver tissues were collected from rats, washed with saline, then they were cut into three small pieces. The first part was fixed in formalin-glutaraldehyde ($_4F_1G$) for ultrastructural examinations and the second part was fixed in 10% neutral formalin for histological examinations while the third part was stored at –80°C until used for the cytogenetic study (comet assay).

Lipid profile tests

Cholesterol and triglycerides levels were analyzed by using diagnostic kits according to Burtis *et al.* [17].

Histological examinations

Liver tissues from the rats of all groups were fixed in 10% neutral formalin and processed for paraffin embedding. Sections of 4-micron thickness were cut by using a rotary microtome and stained with hematoxylin and eosin [18]. Stained sections were examined under a digital light microscope (Olympus, Tokyo, Japan). A minimum of 10 fields for each slide was examined and scored semi-quantitatively for the severity of changes. The scoring was done as none (−), mild (+), moderate (++), and severe (+++) changes [19].

Ultrastructural studies

Liver tissues from rats of all groups were fixed in $_4F_1G$, then rinsed in phosphate buffer solution (pH 7.2) at 4°C for 3 h. Specimens were then post fixed for 2 h in 2% osmium tetroxide and then the specimens were washed with phosphate buffer several times for 10 min. Specimens were dehydrated in a graded ethanol series, followed by propylene oxide, and embedded in epon-araldite mixture in labeled beam capsules. Ultrathin sections were cut using glass knives, collected on naked copper-mesh grids and stained with uranyl acetate for 20 min and lead citrate for 20-30 min [20]. The sections were examined and viewed using Jeol 100CX transmission electron microscopy, Faculty of Science, Alexandria University, Alexandria, Egypt. Thirty cells from each specimen were evaluated by using a previously described scoring system [21].

Cytogenetic study (Comet assay)

Comet assay technique or single cell gel electrophoresis assay detects and evaluates single or double-strand breaks measured at the individual hepatocytes. A damaged DNA containing single-cell suspension was embedded in low melting agarose, lysed by detergents under pH >13 alkaline conditions, then it was electrophoresed for damage displaying which displays any increased emigration of the DNA from the nucleus towards the anode and finally it was neutralized and stained with ethidium bromide. The DNA damage that is made of ethidium bromide-stained DNA was visualized by fluorescent microscope, Faculty of Science, Cairo University, Cairo, Egypt. DNA damage was assessed quantitatively in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Fifty spots of DNA were investigated and categorized into three types: 1) an ordinary spot was spherical shape, 2) destroyed spots in which the length of the migrated fragments was less than or equal to the diameter of the basal nuclear DNA and 3) the strongly damaged spot where the length of DNA migration was more than the diameter of the primary nuclear DNA [22].

Statistical analysis

The present data are presented as mean ± SE and analyzed using the one-way ANOVA. Statistical significance between the experimental groups was assessed by the least significant difference post hoc test, with $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$. The results were computed statistically by using SPSS software package (version 8).

RESULTS

External animal behavior

During the experimental time, rats treated with CLB revealed general weakness, loss of appetite and disability for walking. On the other hand, animals in the other groups did not show any specific behavioral changes.

The change in body weights of rats of the different experimental groups

There was insignificant difference between the initial body weights of rats in the different studied groups. In addition, there was insignificant difference in the final body weights of both moringa oil administered rats and CLB and moringa oil administered rats, comparing with the control group. On the other hand, a significant decrease ($p<0.001$) was recorded in the final body weights of CLB administered rats comparing with the control group. Moreover, there was a significant increase ($p<0.01$) in the final body weights of CLB and moringa oil-treated rats comparing with the final body weights of CLB administered rats (Fig. 1).

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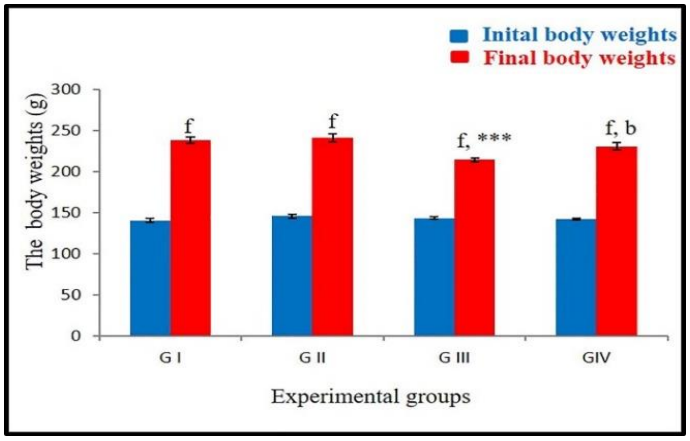

Figure 1: The change in body weights of rats of the different experimental groups.
Control (GI), Moringa oil (GII), CLB (GIII), CLB & Moringa oil (GIV).
(f): final body weight is significant comparing with initial body weight for the same group.
(***): highly significant at $p<0.001$ comparing with control group.
(b): significant at $p<0.01$ comparing with chlorambucil group.

Lipid profile tests

Serum cholesterol and triglycerides levels

Cholesterol and triglycerides levels were represented in figure 2. Moringa oil represented a significant decline ($p<0.05$) in both cholesterol and triglycerides levels as compared with the control rats. A great significant elevation ($p<0.001$) was recorded in the levels of cholesterol and triglycerides in CLB treated rats as compared with the control rats. When rats were received CLB and moringa oil, the levels of both cholesterol and triglycerides showed a highly significant decline ($p<0.001$) comparing with CLB treated rats although a significant elevation ($p<0.01$) was still recording comparing with the control rats.

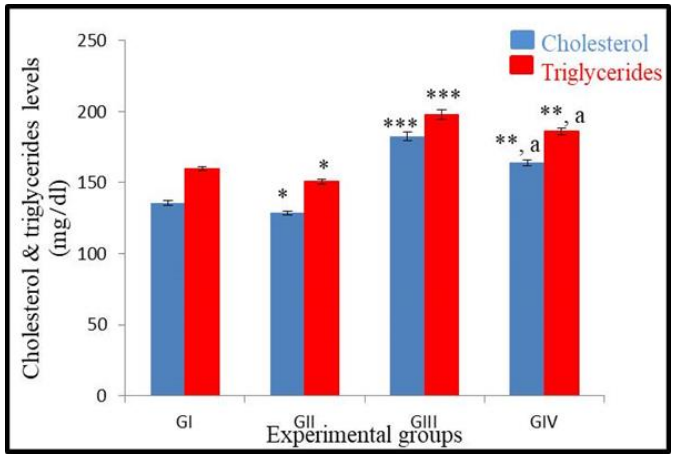

Figure 2: Serum cholesterol and triglycerides levels of the different experimental groups.
Control (GI), Moringa oil (GII), CLB (GIII), CLB & Moringa oil (GIV).
n = 10 animals for each group.
(***): highly significant at $p<0.001$ comparing with control group.
(**): significant at $p<0.01$ comparing with control group.
(*): significant at $p<0.05$ comparing with control group.
(a): highly significant at $p<0.001$ comparing with CLB group.

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Histological observations

The histological examination of liver sections of the control rats and moringa oil treated rats showed normal liver tissue architecture. Normal polygonal hepatic lobules and distinct portal triads were seen. The hepatocytes are arranged in a cord-like fashion and they are separated by blood sinusoids. The central veins were clearly seen (Figs. 3A&B). In the present study, the administration of chlorambucil resulted in some histopathological changes in the liver. The basic structural components of the hepatic lobules were harmed with faded cord-like arrangement of liver cells. Most of the hepatocytes showed vacuolated cytoplasm and pyknotic nuclei. Furthermore, dilation and severe congestion of blood vessels, leukocytic infiltration, activated Kupffer cells and proliferated bile ductules were observed (Figs. 3C&D). Liver sections of rats treated with chlorambucil and moringa oil indicated an obvious degree of improvement; the tissue damage was less extent in this group than the chlorambucil group (Fig. 3E). The observed histopathological changes in all the experimental groups were graded and summarized in table 1.

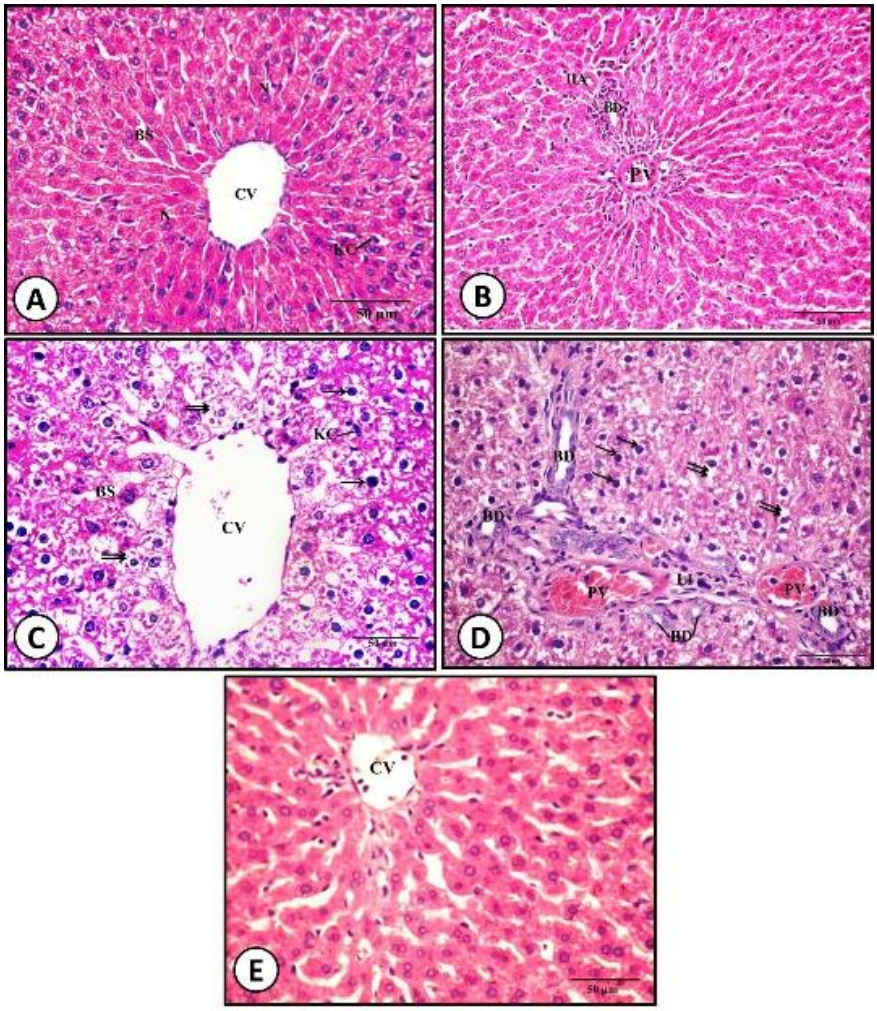

Figure 3A-E: Photomicrographs of hematoxylin and eosin-stained sections of the control and treated groups. A & B): control and moringa oil treated rats, respectively, showing normal liver architecture, central vein (CV), hepatocytes with normal round nuclei (N), blood sinusoid (BS), Kupffer cell (KC), bile ductule (BD), portal vein (PV) and hepatic artery (HA). **C & D):** chlorambucil treated group showing central vein (CV), hepatocytes with pyknotic nuclei (single arrows) and vacuolated cytoplasm (double arrows), dilated blood sinusoid (BS), proliferated bile ductules (BD), leukocytic infiltration (LI) and activated Kupffer cell (KC). **E):** chlorambucil and moringa oil treated group showing improved liver architecture with normal central vein (CV).

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Table 1: Analysis of the observed histopathological and ultrastructural changes in liver tissues of all the experimental groups.

Parameters	Control (GI)	Moringa oil (GII)	CLB (GIII)	CLB & Moringa oil (GIV)
Histopathological changes				
Irregular architecture	–	–	+++	–
Epithelium dislocation	–	–	++	–
Blood vessel dilation	–	–	++	–
Leukocytic infiltration	–	+	+++	–
Pyknotic nuclei	–	–	+++	+
Vacuolar degeneration	–	–	+++	–
Congestion	–	+	++	+
Ultrastructure assessment				
Plasma membrane disruption	–	–		+
Nucleus shrinking	–	–	+++	–
Nuclear envelope preservation	+++	+++	+	+++
Pyknotic nuclei	–	–	++	–
Vacuolated Mitochondria	–	–	+++	+
swelling Mitochondria	–	–	+++	+
RER dilatation	–	–	++	–
Multinucleated hepatocytes	+	+	+++	+

Severity of liver histological changes using scores on a scale of none (–), mild (+), moderate (++), and severe (+++) damage.

Ultrastructural observations

Transmission electron micrographs of sections of control and moringa oil treated animals revealed that the hepatocytes were surrounded by a thin membrane, the plasma-lemma or plasma membrane, which clearly defined their boundaries. The hepatocytes were often attached to each other by junctional complexes, along the plasma membrane. Several bile canaliculi appeared between two or three adjacent hepatocytes as sequestered spaces. These canaliculi were lined with numerous thread-like structures of microvilli which projected into their lumen. Each hepatocyte contained a spherical nucleus surrounded by a regular nuclear envelope. The nucleoplasm showed a normal distribution of heterochromatin and euchromatin. The cytoplasm of the hepatocytes contained numerous evenly distributed round or oval mitochondria. Rough endoplasmic reticulum (RER) was observed as flattened membranes-enclosed cisternae, which have ribosomes attached to it, forming parallel arrays diffused within the cytoplasm (Figs. 4A&B). Smooth endoplasmic reticulum (SER) usually appeared in the form of small vesicles associated with glycogen particles in the cytoplasm.

Furthermore, hepatocytes of rats received chlorambucil revealed a variation in their ultrastructure; some of them lost their polarity and displayed a considerable degree of polymorphism. The plasma membranes showed distinct degenerative features. Bile canaliculi were seen with degenerated microvilli. Marked changes were observed in the nuclei of the hepatocytes; some nuclei appeared with irregular nuclear envelope, sometimes forming nuclear pockets and contained prominent compact and frequently peripheral segregated nucleoli (Fig. 4C). Pyknotic nuclei were

commonly seen in these specimens (Fig. 4D). The morphometric measurements of the nuclear diameters of hepatocytes represented a significant decline (p<0.01) as compared to the control group (Fig. 5A). The majority of mitochondria had excessively dense matrices while their measurements revealed a highly significant increase (p<0.001) in both length and width compared with the control group (Fig. 5B).

Different lipid droplets were scattered in the cytoplasm of some hepatocytes and large lipid droplets were also seen inside the mitochondrial matrix (Figs. 4D &E). Rough endoplasmic reticulum showed disorganization and dilation of their cisternae. The blood sinusoids contained activated Kupffer cells with highly irregular nuclei (Fig. 4E).

Electron microscopic examination of liver cells of rats treated with chlorambucil and moringa oil revealed a marked degree of recovery compared with liver cells of animals treated with chlorambucil. The hepatocytes were closely associated together and most of them maintained their normal polygonal shape. They were surrounded by regular and intact cell membranes and contained relatively rounded nuclei with regular nuclear envelopes. The microvilli of both spaces of Disse and bile canaliculi were normal and well developed (Fig. 4F). The morphometric measurements of the nuclear diameters of hepatocytes showed a significant increase (p<0.05) comparing with chlorambucil administered group and an insignificant difference when comparing with the control group (Fig. 5A). The cytoplasm of the hepatocytes showed regular organization and normal distribution of the cellular organelles with no detectable morphological alterations. The morphometric measurements of mitochondrial dimensions showed a significant reduction (p<0.001 and p<0.01) in length and width, respectively, comparing with chlorambucil treated group and insignificant difference comparing with the control group (Fig. 5B). Table 1 summarized the ultrastructural changes of the liver tissues score according to severity.

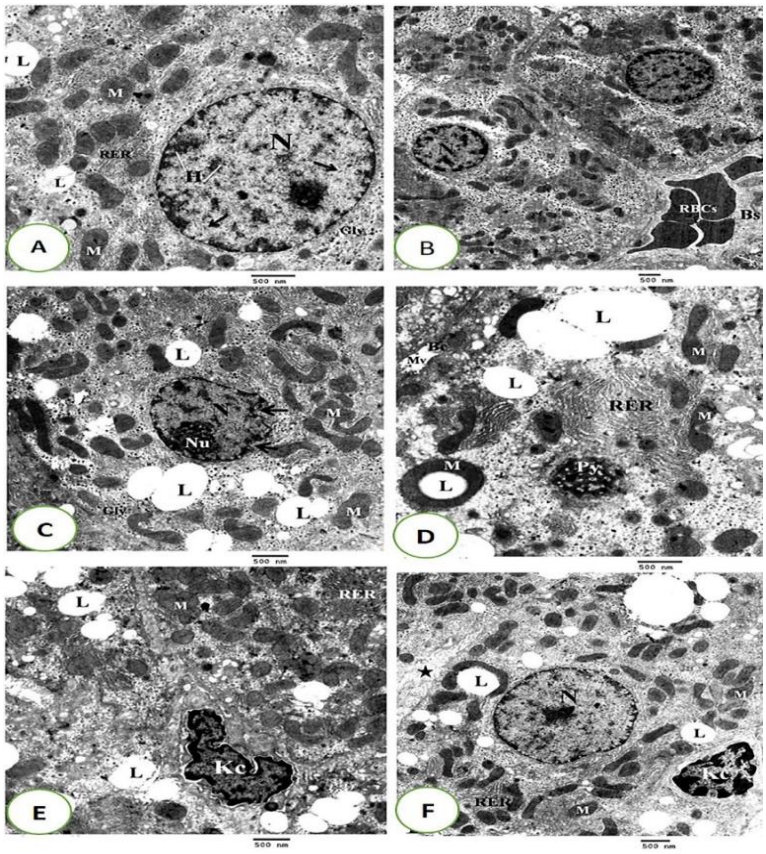

Figure 4A-F: Transmission electron micrographs of hepatocytes of the different experimental groups. **A&B):** hepatocytes of control and moringa oil treated animals, respectively, showing normal hepatocytes with spherical nucleus (N) and regular nuclear envelope, normal heterochromatin (H), euchromatin (arrows), normal mitochondria (M), parallel cisternae of rough endoplasmic reticulum

(RER), microvilli (Mv) of a bile canaliculus, glycogen rosettes (Gly), lipid droplets (L) and blood sinusoid (Bs) contains red blood cells (RBCs). **C-E):** showing hepatocyte of chlorambucil treated rats contains irregular nucleus (N) with nuclear pockets (arrows) and segregated nucleolus (Nu), pyknotic nucleus (Py), polymorphic mitochondria (M), large lipid droplets (L) in the cytoplasm or inside the mitochondria, disorganized and dilated cisternae of rough endoplasmic reticulum (RER), glycogen rosettes (Gly), bile canaliculi (Bc) with damaged microvilli (Mv) and Kupffer cell (Kc) with highly irregular nucleus. **F):** hepatocytes of chlorambucil and moringa oil treated rats showing nucleus (N) with regular nuclear envelope, normal mitochondria (M), rough endoplasmic reticulum (RER), lipid droplets (L), Kupffer cell (Kc) and space of Disse (*).

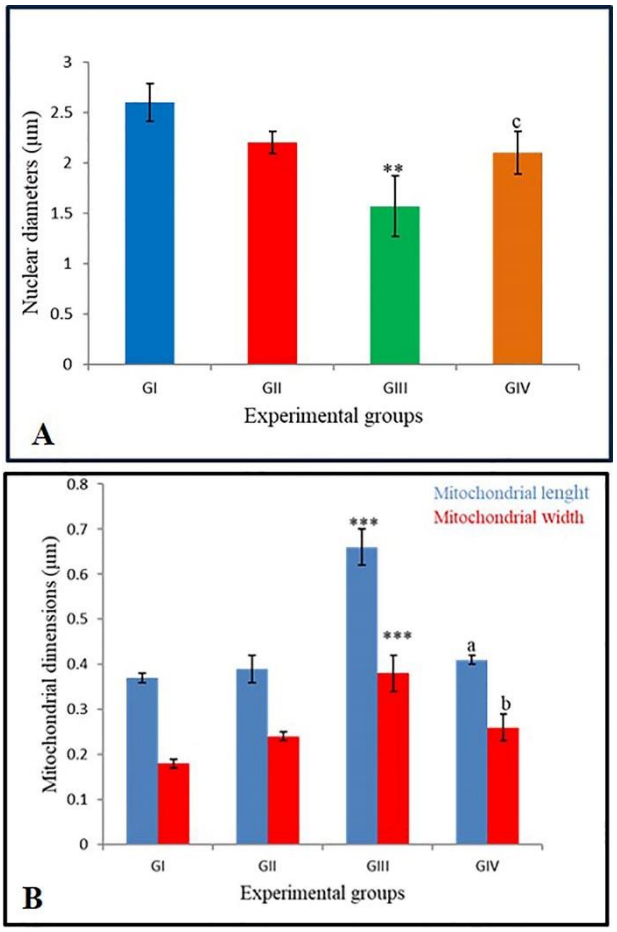

Figure 5A & B: Morphometric measurements, **A):** The hepatocytes nuclear diameters. **B):** The mitochondrial dimensions (length and width) of the different experimental groups.
Control (GI), Moringa oil (GII), CLB (GIII), CLB & Moringa oil (GIV).
(***): highly significant at $p<0.001$ comparing with control group.
(**): significant at $p<0.01$ comparing with control group.
(a): highly significant at $p<0.001$ comparing with chlorambucil group.
(b): significant at $p<0.01$ comparing with chlorambucil group.
(c): significant at $p<0.05$ comparing with chlorambucil group.

Cytogenetic observations

Data obtained from comet assay; tail length, DNA percent in the tail and tail moment were represented in table 2 and figure 6. The nuclei of hepatocytes of the control rats showed normal DNA rounded spots without migration towards the anode. Also, the nuclei of hepatocytes of rats treated with moringa oil exhibited normal DNA spots with an insignificant difference in tail length, DNA percent in tail and tail moment comparing with the control group (Figs. 6A, B &E). On the other hand,

the hepatocytes of animals treated with chlorambucil showed damaged DNA tailed spots with greatly significant elevation (p<0.001) in DNA tail length, DNA percent in tail and tail moment compared with the control group (Figs. 6C&E). When animals treated with chlorambucil and moringa oil, the nuclei of hepatocytes showed slightly damaged DNA short-tailed spots with significant decrease in tail length, tail DNA% and tail moment (p<0.001, p<0.05 and p<0.001, respectively) compared with chlorambucil group but it still showing a significant increase in DNA percent in the tail (p<0.01) comparing with the control group (Figs. 6D&E).

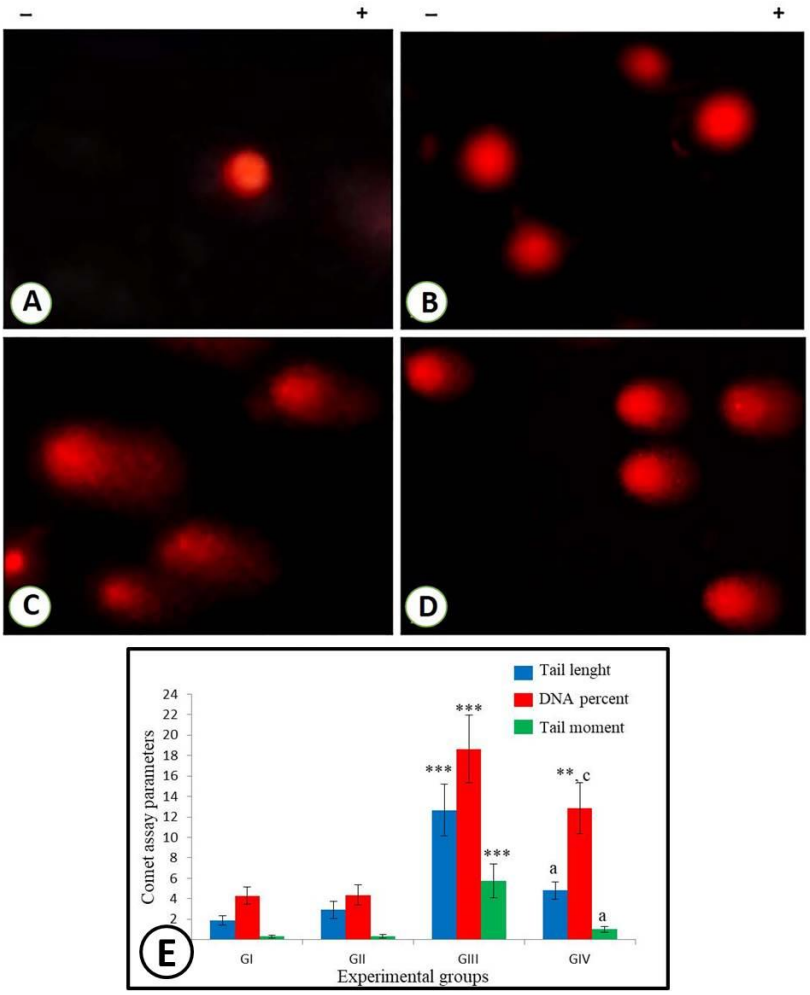

Figure 6A-E: DNA fragmentation in the hepatocytes of all the experimental groups: A &B): Control and moringa oil groups, respectively, showing normal DNA round spot with no migration of nuclear DNA. **C):** Chlorambucil group showing severe signs of DNA fragmentation with strongly damaged DNA tailed spots with strong migration towards the anode. **D):** Chlorambucil and moringa oil group showing slightly damaged DNA short tailed spots with slight migration towards the anode. **E):** Graphical representation of comet assay parameters; tail length, DNA percent in tail and tail moment in rat hepatocytes of the different experimental groups. Control (GI), Moringa oil (GII), CLB (GIII), CLB & Moringa oil (GIV).

(***): highly significant at p<0.001 comparing with control group.

(**): significant at p<0.01 comparing with control group.

(a): highly significance at p<0.001 comparing with chlorambucil group.

(c): significant at p<0.05 comparing with chlorambucil group.

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Table 2: Comet assay parameters (Mean ± SE); tail length (px), DNA percent in tail (%) and tail moment in rat hepatocytes of the different experimental groups.

Parameters / Group	Tail length (px)	DNA percent (%)	Tail moment
Control group (GI)	1.88 ± 0.45	4.30 ± 0.85	0.31 ± 0.14
Moringa oil group (GII)	2.90 ± 0.84	4.37 ±0.97	0.36 ± 0.18
Chlorambucil group (GIII)	12.66 ± 2.51***	18.64 ± 3.32***	5.76 ± 1.64***
Chlorambucil & moringa oil group (GIV)	4.80 ± 0.85[a]	12.86 ± 2.5**, c	1.03 ± 0.28[a]

n= 50 hepatocytes nuclei for each group.
(***): highly significant at $p<0.001$ comparing with control group.
(**): significant at $p<0.01$ comparing with control group.
(a): highly significance at $p<0.001$ comparing with chlorambucil group.
(c): significant at $p<0.05$ comparing with chlorambucil group.

DISCUSSION

The liver plays an amazing array of vital functions in maintaining body balance, functioning and organization. It is involved with almost all biochemical pathways for growth, disease control, nutrient supply, energy-saving and reproduction [23].

In the present study, rats treated with chlorambucil (1.26 mg/ Kg b.w) daily for four weeks showed certain external symptoms and many changes in the general behavior. The major symptoms were loss of appetite, loss of body weight gain and decreased spontaneous activity. The observed weight loss in CLB treated animals may be due to the reduced appetite of these animals throughout the experimental period. The body weight changes occupy a special attention in rats treated with the anticancer drugs. El-Sayyed *et al*. [24] showed a significant decrease in body weight gain after treatment of rats with cisplatin, doxorubicin and 5-flurouracil.

Similarly, El-Gerbed [25] observed decrease in the body weight of rats after cisplatin administration and attributed this loss to the reduced appetite and enhanced catabolic rate which are considered as the obvious side effects of chemotherapy or due to dysfunction of the gastrointestinal system induced by cisplatin.

In the present study, a greatly significant elevation in the levels of cholesterol and triglycerides were recorded in CLB treated animals which may be due to the oxidative stress produced by CLB treatment. In this respect, Fakurazi *et al.* [14] reported that the liver plays a pivotal role in the conservation of systemic lipid internal stability and is susceptible to reactive oxygen spices (ROS) harm.

The treatment with CLB and moringa oil resulted in a significant reduction in the levels of both cholesterol and triglycerides. This reduction may be attributed to the antioxidant properties of moringa oil. Moringa oil has therapeutic effects in terms of hypocholesterolemic effects (due to its content of phytosterols) and was capable of scavenging free radicals (due to the presence of tocopherols, phenolics and carotenoids) [26]. Similar results were reported by Neveda *et al.* [27] after

the treatment of rats with moringa leaves extract and Adeyemi *et al*. [28] after using moringa leaves as a part of the diet with nickel induced hepatotoxicity.

The data of our study also revealed that, treatment of rats with chlorambucil has caused many histological changes including leukocytic infiltration, congestion of blood vessels and widening of the sinusoids. Besides, the results showed intense cytoplasmic vacuolization of the hepatocytes. These findings were in agreement with the previous study on chlorambucil-induced hepatotoxicity in rats [5].

The observed leukocytic infiltration is a sign of the immune response to overcome the toxic effects of CLB or its metabolites. Leukocytic infiltration is an outstanding response to body tissues facing any adverse effects [29]. It is believed that the mechanism of chemotherapy-induced hepatic injury is thought to be secondary to the production of ROS, leading to an impaired ability to regenerate and abnormal innate immunity [30]. In the present study, histological examination of the liver of rats treated with CLB and moringa oil showed a marked degree of recovery which can be attributed to the antioxidant properties of moringa oil components. In agreement with the present result, moringa leaves extract improved the liver histological changes induced by methotrexate in rats [31].

In the present study, obvious ultrastructural changes in the liver of CLB treated rats were seen. The nuclei of hepatocytes appeared with irregular nuclear envelopes; sometimes nuclear pockets were formed. The nuclear diameters significantly decreased compared with that of the control rats. Also, some pyknotic nuclei and segregated nucleoli were observed. Nuclear irregularity provides an expanded nuclear surface area, and allow for the increased nucleocytoplasmic exchange and metabolic activity [32]. The nucleolus pass obvious structural alteration and molecular order when incurring to toxic compounds cells [33]. Chemotherapeutic drugs first and foremost inhibit ribosomal RNA synthesis and processing which related to the formation of segregated nucleoli [34].

In the current study, as a result of CLB treatment, mitochondria appeared polymorphic and contained highly dense matrices. Moreover, a significant increase in their dimensions was recorded. Also, dilation of endoplasmic reticulum and distortion or fragmentations of microvilli of both spaces of Disse and bile canaliculi were observed. The mitochondrial damage in the current results may be due to the liberation of ROS which can result in oxidative stress and consequently cytoplasmic organelles damage. Mitochondria play an important role in several functions including growth, division, energy metabolism, and apoptosis [35].

In hepatocytes of CLB treated rats, numerous lipid droplets were seen which may be also as a result of mitochondrial dysfunction. Increased intracellular lipid considered as a protective mechanism by which liver cells try to gather most toxic compounds that invade the cell in these gaps before they are secreted [36]. During lipid peroxidation caused by ROS, mitochondria are predominantly associated with oil droplets containing fatty acids from which they derive raw materials for oxidation, lipid synthesis and metabolism that takes place primarily in the mitochondria [37].

In the current study, activated Kupffer cells with highly irregular nuclei were observed which may be due to the simulative effect of chlorambucil or its metabolites on liver immune-response. Kupffer cells are intensively involved in the endocytosis of foreign agents which results in their activation [38]. They produce an array of mediators that protect the liver against invasion by xenobiotic and chemical materials [39] .

In the present study, an improvement in the ultrastructural changes of liver tissue of rats treated with CLB and moringa oil was observed. The hepatocytes were closely associated together, maintained their normal polygonal shape and exhibited normal numerous microvilli of both spaces of Disse, and bile canaliculi. The hepatocytes nuclei appeared round with regular nuclear envelopes and showed a significant increase in their diameters comparing with that of the CLB treated rats. Mitochondria normally appeared and showed a significant decrease in their dimensions comparing with that of CLB group. Rough endoplasmic reticulum with normal cisternae and less abundant lipid

droplets were observed. This improvement may be due to the antioxidant activities of moringa oil components. The antioxidants have been propositioning to play an important role in keeping the physiological levels of oxygen and hydrogen peroxide and elimination of peroxides as a result inadvertent exposure to xenobiotics and drugs [40] Similar findings were reported previously by Taha *et al*. [41] who reported that *Moringa oleifera* leaves improved the liver ultrastructural damage induced by diclofenac sodium in rats.

Cytogenetically, by using the comet assay, hepatocytes of CLB treated rats revealed significant DNA damage. This DNA damage may be a result of the formation of a highly reactive ethylenimonium radical [5] which is known to result in breaks in the DNA molecule as well as cross-linking of the DNA double strands and consequently interfering with DNA replication and transcription of RNA [42]. The resultant structural and functional damage to DNA leads to the cytotoxic and first step in the development of cancer [5]. Moringa oil treatment significantly decreased DNA damage induced by CLB which may be due to free radical scavenging potency of moringa oil. These results were in agreement with Sikder *et al*. [43] who reported that moringa leaves extract directly prevented the hydroxyl radical-induced DNA damage in mice lymphocytes.

CONCLUSION

The present study elucidated the therapeutic effects of moringa oil administered in combination with chlorambucil to minimize its liver injury, and its effects may intercede at least partly by the suppression of DNA damage through scavenging reactions of free radicals.

List of abbreviations

Chlorambucil (CLB), *Moringa oleifera* (MO), Transmission electron microscopy (TEM).

Ethical approval

Permission and approval for an animal (rats) studies were obtained from the Faculty of Science, University of Menoufia, Egypt (Approval No. MNSH180).

Data availability

Data will be made available upon reasonable request to the corresponding author.

REFERENCES

[1] Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer*, 2015; 136(5), E359-E386.

[2] Florea, A.M.; Büsselberg, D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. *Cancers*, 2011; 3, 1351-1371.

[3] Guainazzi, A.; Schärer, O.D. Using synthetic DNA interstrand crosslinks to elucidate repair pathways and identify new therapeutic targets for cancer chemotherapy. *Cell Mol. Life Sci.,* 2010; 67, 3683-3697.

[4] Salem, F.S.; Badr, M.; Neamat-Allah, A. Biochemical and pathological studies on the effects of levamisole and chlorambucil on Ehrlich ascites carcinoma-bearing mice. *Vet. Ital.*, 2011; 47, 89-95.

[5] Olayinka, E.; Ore, A.; Fashiku, K.A. Kolaviron and L-ascorbic acid ameliorates chlorambucil-induced hepatic and renal toxicity in rat. *Int. J. Toxicol. Appl. Pharm.,* 2014; 4, 23-32.

[6] Yousef, M.I.; Omar, S.A.; El-Guendi, M.I.; Abdelmegid, L.A. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem. Toxicol.,* 2010; 48, 3246-3261.

[7] Arora, D.S.; Onsare, J.G.; Kaur, H. Bioprospecting of Moringa (Moringaceae): microbiological perspective. *J. Pharmacog. Phytochem.,* 2013; 1, 193-215.

[8] Vaknin, Y.; Mishal, A. The potential of the tropical "miracle tree" *Moringa oleifera* and its desert relative Moringa peregrina as edible seed-oil and protein crops under Mediterranean conditions. *Sci. Hortic*., 2017; 225, 431-437.

[9] Anwar, F.; Bhanger, M. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *J. Agric. Food Chem*., 2003; 51, 6558-6563.

[10] Moyo, B.; Oyedemi, S.; Masika, P.; Muchenje, V. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Sci.,* 2012; 91, 441-447.

[11] Hamza, A.A. Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. *Food Chem. Toxicol*., 2010; 48, 345-355.

[12] Olatosin, T.; Akinduko, D.; Uche, C.; Bardi, J. Effects of *Moringa oleifera* seed oil on acetaminophen-induced oxidative stress and liver damage in Wistar albino rats. *IOSR J. Pharm. Biol. Sci.,* 2014; 9, 53-59.

[13] Omabe, M.; Omabe, K. N.; Igwe, D.; John, O. C.; Uchenna, S. K.; Elom, S. Xenobiotics-induced liver damage is biochemically abrogated by treatment with lipophilic extracts of *Moringa oleifera In vivo*. *Health*, 2018; 10(3), 313-325.

[14] Fakurazi, S.; Sharifudin, S.A.; Arulselvan, P. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules*, 2012; 17, 8334-8350.

[15] Ruttarattanamongkol, K.; Siebenhandl-Ehn, S.; Schreiner, M.; Petrasch, A.M. Pilot-scale supercritical carbon dioxide extraction, physico-chemical properties and profile characterization of *Moringa oleifera* seed oil in comparison with conventional extraction methods. *Ind. Crops Prod*., 2014; 58, 68-77.

[16] Laurence, D.R.; Bacharach, A.L. *Evaluation of drug activities: pharmacometrics*. 2013: Elsevier.

[17] Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. *Tietz textbook of clinical chemistry and molecular diagnostics-e-book*. 2012: Elsevier Health Sciences.

[18] Cook, H.C.; Stirling, R. *Manual of histological techniques and their diagnostic application*. 1994: Churchill Livingstone.

[19] Suzuki, H.; Suzuki, K. Rat hypoplastic kidney (hpk/hpk) induces renal anemia, hyperparathyroidism, and osteodystrophy at the end stage of renal failure. *J. Vet. Med. Sci.,* 1998; 60, 1051-1058.

[20] Reynolds E.S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 1963; 17, 208-212.

[21] López-Alonso, B.; Hernáez, A.; Sarnago, H.; Naval, A.; *et al.* Histopathological and ultrastructural changes after electroporation in pig liver using parallel-plate electrodes and high-performance generator. *Sci. Rep.,* 2019; 9, 1-12.

[22] Singh, N.P.; McCoy, M.T.; Tice, R.R.; Schneider, E.L. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.,* 1988; 175, 184-191.

[23] Sharma, A.; Chakraborti, S.K.; Handa, S.S.; Chakraborti, K.; *et al.* Anti-hepatotoxic activity of some Indian herbal formulations as compared to silymarin. *Fitoterapia,* 1991; 62, 229-235.

[24] El-Sayyad, H. I.; Ismail, M. F.; Shalaby, F. M.; Abou-El-Magd, R. F.; Gaur, R. L.; Fernando, A.; Raj, M. H. G.; Ouhtit A. Histopathological effects of cisplatin, doxorubicin and 5-flurouracil (5-FU) on the liver of male albino rats. *Int. J. Biol. Sci.,* 2009; 5(5), 466-473.

[25] El-Gerbed, M. S. A. Ameliorative effect of fish oil on the cisplatin induced hepatotoxicity and nephrotoxicity in rats. *Res. J. Pharm. Biol. Chem. Sci*., 2013; 4, 479-491.

[26] Bhatnagar, A.; Krishna, A.G. Natural antioxidants of the Jaffna variety of *Moringa Oleifera* seed oil of Indian origin as compared to other vegetable oils. *Grasas Aceites,* 2013; 64, 537-545.

[27] Neveda, O.; Asna, U.; Preetham Paul, P.; Narayan Prasad, N. Effect of dietary lipids and drumstick leaves (*Moringa oleifera*) on lipid profile & antioxidant parameters in rats. *Food Sci. Nutr.,* 2012; 2012.

[28] Adeyemi, O.S.; Aroge, C.S.; Akanji, M.A. *Moringa oleifera*-based diet protects against nickel-induced hepatotoxicity in rats. *J. Biomed. Res*., 2017; 31, 350-357.
[29] Sakr, S.; Azab, A. Effect of pyrethroid inhalation on the testis of albino rat. *PJBS,* 2001; 4, 498-500.
[30] Joshi, M.; Sodhi, K.S.; Pandey, R., Singh, J.; *et al.* CANCER CHEMOTHERAPY AND HEPATOTOXICITY: AN UPDATE. *J. Pharm. Res.,* 2014; 4.
[31] Yousef, F.M.A.; Khattab, H.A.R.H.; Sindi, H.A.A. Effectiveness of *Moringa oleifera* L. Leaves Extract Against Methotrexate-induced Acute Hepatotoxicity in Male Rats. *Int. J. Pharmacol.,* 2018; 14, 1029-1037.
[32] de Brito-Gitirana, L.; Miguel, N. Electron microscopical investigation on aldrin-induced hepatocyte pathology in Rana catesbeiana, with special emphasis on peroxisomes. *Exp. Toxicol. Pathol.,* 2000; 52, 339-347.
[33] Boulon, S.; Westman, B.J.; Hutten, S.; Boisvert, F.M.; *et al.* The nucleolus under stress. *Mol. Cell,* 2010; 40, 216-227.
[34] Burger, K.; Mühl, B.; Harasim, T.; Rohrmoser, M.; *et al.* Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. *J Biol. Chem.,* 2010; 285, 12416-12425.
[35] Alirol, E.; Martinou, J.C. Mitochondria and cancer: is there a morphological connection? *Oncogene*, 2006; 25, 4706-4716.
[36] AlJahdali, M.O.; Bisher, A.S.B.; Zeid, I.M.A. Physiological and Histological Alterations in Rats Liver Induced by Sumithion NP 25/2.5 EC, an Insecticide Used in Dengue Fever Vector Control in Jeddah, Saudi Arabia. *Saudi J. Biol. Sci.*, 2009; 14, 43-51.
[37] Xu, W.N.; Liu, W.B.; Liu, Z.P. Trichlorfon-induced apoptosis in hepatocyte primary cultures of Carassius auratus gibelio. *Chemosphere,* 2009; 7, 895-901.
[38] Kolios, G.; Valatas, V.; Kouroumalis, E. Role of Kupffer cells in the pathogenesis of liver disease. *World J. Gastroenterol.,* 2006; 12, 7413-7420.
[39] Sadauskas, E.; Danscher, G.; Stoltenberg, M.; Vogel, U.; *et al.* Protracted elimination of gold nanoparticles from mouse liver. *Nanomed. Nanotechnol.,* 2009; 5, 162-169.
[40] Paliwal, R.; Sharma, V.; Pracheta, S.S.; Yadav, S.; *et al.* Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. *Biol.Med.*, 2011; 3, 27-35.
[41] Taha, N.R.; Rabah, S.O.; Shaker, S.A.; Mograby, M.M. Effect of *Moringa oleifera* leaves on diclofenac sodium induced hepatic injury in albino rats: ultrastructural and immunohistochemical studies. *J. Cytol. Histol.,* 2015; 6, 1.
[42] Li, W.X.; Yan, X.; Shi, C.R.; Zhang, A.P. Chlorambucil for patients with primary biliary cirrhosis. *Cochrane Database Syst. Rev.,* 2012.
[43] Sikder, K.; Sinha, M.; Das, N.; Das, D.; *et al.* *Moringa oleifera* Leaf extract prevents in vitro oxidative DNA damage. *Asian J. Pharm. Clin. Res.* 2013; 6, 157-161.