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# Evaluation of Protective and Therapeutic Role of *Moringa oleifera* leaf extract on $CCL_4$-induced genotoxicity, hemotoxicity and hepatotoxicity in rats

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**Abstract:** The present study was designed to evaluate the protective and therapeutic effect of *Moringa oleifera* leaf extract (MOLE) against carbon tetrachloride ($CCL_4$)-induced genotoxicity, hemotoxicity and hepatotoxicity in rats. Male albino rats of eleven groups (eight animals each) were used in this study. The animal groups included negative control; control of olive oil; positive control (received $CCL_4$ in olive oil for 12 weeks); Groups 4-7 received $CCL_4$ in olive oil plus MOLE at doses of 1.3, 2.0, 2.6 and 4.0 g/kg (used as a protective agent) for 12 weeks; Groups 8-11 received MOLE alone (as a therapeutic agent) at the same doses for 3 weeks after cessation (12 weeks) of $CCL_4$ treatment. Molecular genetic, hematological, histopathological and histochemical studies were conducted. Genetic results showed that the administration of $CCL_4$ caused a high significant increase of DNA damage in lymphocyte cells and significant elevation of the expression of CYP1A2 and CYP2B1 genes in liver tissue as compared to control. Also hematological findings revealed that $CCL_4$ treatment significantly reduced Hb level and RBCs count, whereas it significantly increased the WBCs count in respect to normal control. Histopathological examination documented that $CCL_4$ produced massive damage to liver tissue in the form of excessive fibrosis, cellular infiltration and vacuolar degeneration of hepatocytes. MOLE treatment (as a protective or therapeutic agent) was able to significantly reduce the DNA damage and significantly inhibit the up-regulation of CYP1A2 and CYP2B1 genes expression induced by $CCL_4$. It also significantly improved the hematological parameters, where the abnormal changes in Hb level, RBCs count and WBCs count induced by $CCL_4$ had been minimized. Moreover, the histopathological results revealed that the damaging effect of $CCL_4$ on hepatic tissue was clearly reduced by using MOLE treatment. Histochemical findings confirmed the histopathological results, where the DNA study indicated that MOLE treatment ameliorated the DNA content in examined cells and gave DNA values better than those of animals group treated with $CCL_4$ alone. While the $CCL_4$ group showed decrease of DNA values (hypoploidy). All the above results were dose dependent. But better results were obtained by using MOLE as a therapeutic agent, especially the treatment with the highest dose 4.0 g/kg, in which the rate of DNA damage, the overexpression especially of CYP1A2 gene, hematological changes and the massive damage in liver tissue as well as the abnormal histochemical parameters reverted nearly to the normal values.
In conclusion, the present study proved that MOLE is able to significantly alleviate the oxidative stress induced by $CCL_4$ in rats., These results revealed that *Moringa oleifera* has therapeutic effect in curing some health problems associated with toxication status (as a result of $CCL_4$ treatment) and this was established by its positive effect on some of molecular genetic, hematological, histopathological and histochemical parameters of the experimental animals.
**Keywords:** *Moringa oleifera* leaf extract, $CCL_4$-induced genotoxicity, hemotoxicity, hepatotoxicity, rats.

## Introduction

Liver disorders are one of the serious health problems through the world. Despite remarkable advances in the field of modern medicine, hepatic diseases remain a major public health problem, thus the search for new effective medicines without side effects is still ongoing [1]. Natural remedies from traditional plants are seen as effective and safe alternative treatments for hepatotoxicity [2-4]. Several studies showed that hepatoprotective effects of medicinal plants are associated with phytoextracts/phytocompounds rich in natural antioxidants [2, 5-7] Many phytochemicals are strong antioxidants, effective antimicrobials, possess substantial anticarcinogenic and antimutagenic properties [8-10], as well as they are also active in reducing high blood pressure [11-13].

*Moringa oleifera Lam* is the most widely distributed species of the Moringaceae family throughout the world, especially in Asian and African countries, having a remarkable range of pharmacological properties in addition to significant nutritional value [14, 4]. The various plant parts have wide medicinal applicability for the treatment of cardiovascular diseases. The roots, leaves, gum, flowers and seed infusion contain nitrile, mustard oil glycosides and thiocarbamate glycosides as their important bioactive constituents, which are thought to be responsible for their diuretic, cholesterol lowering and antiulcer properties [15, 4]. Moreover, seed pod extracts have been demonstrated to prevent skin tumors in mice [16]. On the other hand, the extract of Moringa leaves has been shown to have potent antioxidant action *in vivo* [17,18]. Because this part of plant when compared to other parts, it was found that it is high significant source of protein, β-carotene, vitamins A,B,C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds [15, 14, 4]. Also, *Moringa oleifera* leaves were used as nutritional supplement and growth promoters [19-23]. The extract of this part of the plant has been investigated to be a protective or therapeutic agent against various abnormal conditions. Ethanolic extract of leaves has shown antimicrobial activity [24, 25]. Radio protective effect of leaves has also been established where in radiation-induced chromosomal aberrations and micronuclei were suppressed by pre-treatment with methanolic extract [26]. Moreover, *M. oleifera* leaves aquous extract was observed to have a therapeutic action against radiation hazards through enhancing of liver enzyme activities (AST, ALT and ALK), decreasing the malondialdehyde (MDA), and reduction of genetic alterations (micronuclei and DNA damage) in irradiated rats by gamma irradiation [27]. Furthermore, ethanolic extract of *M. oleifera* leaves possessed antigenotoxic phytoconstituents in mice, the high percentages of micronuclei and DNA damage induced by cyclophosphamide were minimized in animals pre-dosed with the extract [28]. On the other hand, the treatment with ethanolic extract of M. oleifera leaves effectively protected hepatic tissue from tissue damage induced by antitubercular drugs [29].

Moringa leaves contain a rich and rare combination of zeatin, quercetin and Kaempferom that have shown potent anticancer, anti-inflammatory, hepatoprotective and antifungal activities [30, 31].

Carbon tetrachloride ($CCL_4$) has been used in animal model to induce liver damage similar to that of acute viral hepatitis in human patients [32]. The principle causes of carbon tetrachloride in inducing the hepatic damage are lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals [33, 32]. Also, this component ($CCL_4$) is a commonly used model for screening of the anti-hepatotoxic and/or hepatoprotective activities of the drugs [34-36]. So, the present study was designed to evaluate the protective role and therapeutic effect of *Moringa oleifera* leaves ethanolic extract against $CCL_4$-induced hepatotoxicity and genotoxicity in rats. The study was carried out to investigate its effects on gene expression alterations of cytochrome P450 isoforms (CYP1A2 and CYP2B1) and assaying of histopathological and histochemical changes in rat livers. Comet assay of DNA and hematological examinations in blood cells were also analyzed.

## Materials and Methods

### Chemicals:

### Carbon tetrachloride ($CCL_4$):

$CCL_4$ is a colorless non-flammable liquid, of molecular weight 153:84 was obtained from El-Nasr Pharmaceutical chemical Co., A.R.E.

$CCL_4$ is one of the most commonly used hepatotoxins in the experimental studies of liver diseases. This compound induced hepatotoxicity as judged from serum marker enzymes and antioxidant levels in liver tissues [37].

**Preparation of *Moringa oleifera* leaves extract (MOLE):**

Ethanolic extract of *Moringa oleifera* leaves was prepared according to the method of Ugwu *et al*. [38], as follows: The fresh leaves of *Moringa oleifera* plant were picked from trees grown on sand soil in El-Sharkia governorate, Egypt. The leaves were washed thoroughly with distilled water and dried under room temperature at (29°C-35°C) for three weeks, after which the leaves were pulverized into coarse form with acrestor high speed milling machine. The coarse form (1000 g) was then macerated in absolute ethanol. This was left to stand for 48h. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature was between 40 and 45°C to avoid denaturation of the active ingredients. The concentrated extract was diluted to 1000 ml using a polysaccharide as a carrier and stored in the refrigerator.

**Experimental animals:**

Male albino rats of Sprague-Dawley strain weighing 120-150 g were obtained from the animal house, National Research Centre, Egypt. Animals were housed in an ambient temperature of 25 $\pm$ 3.2°C on light/dark cycle of 12/12 hours. All rats were kept in clean polyprophylene cages and administered food and water *ad libitum*.

**Experimental design:**

The rats were divided into 11 equal groups, 8 rats each. Group 1, received saline. Group 2, received the vehicle (olive oil) at 2.8 ml/kg. Group 3, received $CCl_4$ in olive oil (1:1, vol/vol) at a dose of 2.8 ml/kg through orogastric tube and then the rats were administered half the initial dose of $CCl_4$, twice weekly after the first administration of $CCl_4$ for 12 weeks so as to maintain hepatic damage. Group 4-7, received $CCl_4$ in olive oil in the same dose and way previously mentioned and for the same period. Starting on the first day of $CCl_4$ administration, rats were treated with moringa extract (1.3 g/kg, 2.0 g/kg, 2.6 g/kg, 4.0 g/kg). Groups 8-11, received $CCl_4$ in olive in the same dose and way as previously mentioned and for the same period, then rats were treated with moringa extract (1.3g/kg, 2.0 g/kg, 2.6 g/kg, 4.0 g/kg) for three weeks.

Rats had free access to food and drinking water during the study. At the end of the experiment, blood samples were collected for DNA comet assay and hematological examination. After that, the animals were sacrificed by cervical dislocation. Livers were immediately removed for molecular genetic study and for histopathological and histochemical analyses.

The used doses of $CCL_4$ for rats were quivalent to the human dose according to Paget and Barnes [39] and Abdel Salam *et al*. [40, 41].

**Comet Assay:**

Peripheral blood lymphocytes were isolated by centrifugation (15 min, 280 g) in a density gradient of Gradisol L (Aqua Medica, Lodz, Poland). The concentration of the cells was adjusted to (1-3) x $10^5$ cells/ml by adding RPMI 1640 without glutamine to the single cell suspension. A freshly prepared suspension of cells in 0.75% low melting point agarose (Sigma Chemicals) dissolved in phosphate buffer saline (PBS; Sigma chemicals) was cast onto microscope slides precoated with 0.5% normal melting agrose. The cells were then lysed for 1h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100 and 10 mM Tris, pH 10. After the lysis, DNA was allowed to unwind for 40 min in electrophoretic solution consisting of 300 mM NaOH, 1mM EDTA, pH>13. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73 V/cm (30mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 ug/ml ethidium bromide (Sigma Chemicals) and covered with cover slips. The slides were examined at 200 x magnification fluorescence microscope (Nikon Tokyo, Japan) connected to a COHU 4910 video camera (Cohu, Inc., SanDiego, CA, USA) equipped with a UV vilter block consisting an excitation filter (359 nm) and barrier filter (461nm) and connected to a personal computer-based image analysis system, Lucia-Comet v. 4.51. Fifty images were randomly selected from each sample and the comet tial DNA was measured [42]. Endogenous DNA damage was measured as the mean comet tail DNA of peripheral blood lymphocytes of five mice groups (8 mice each). The number of cells scored for each animal was 100 [42].

**Gene expression assay:**

**Semi-quantitative RT-PCR:**

***RNA extraction:***

Total RNA was isolated from 100 µg of liver tissue samples by the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 µl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol.

**Reverse transcription:**

The complete Poly(A)+ RNA samples were reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo(dT) primer. The composition of the reaction mixture, termed as master mix (MM), consisted of 50 mM MgCl2, 10x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3; Perkin-Elmer), 10 mM of each dNTP (Amersham, Brunswick, Germany), and 50 µM of oligo(dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through polymerase chain reaction (PCR).

**Polymerase chain reaction (PCR):**

The first strand cDNA from different samples was used as templates for RT-PCR with a pair of specific. The sequences of specific primer and product sizes are listed in Table 1. β-Actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM MgCl2, 10x PCR buffer (50 mM KCl; 20 mM Tris-HCl; pH 8.3; Gibco BRL, Eggenstein, Germany), and autoclaved water. The PCR cycling parameters were one cycle of 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 42 °C to 58 °C for 30 s, 72 °C for 90 s, and a final cycle of 72 °C for 7 min. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different samples [43].

**Table 1. Primers and reaction parameters in RT-PCR**

| Target cDNA | Primer name | Primer sequence (5′–3′) | Annealing temperature (°C) | PCR product size (bp) |
|---|---|---|---|---|
| β-Actin | F | CCC CAT CGA GCA CGG TAT TG | 57 | 189 |
| | R | ATG GCG GGG GTG TTG AAG GTC | | |
| CYP1A2 | F | GAT GAG AAG CAG TGG AAA GAC C | 50 | 328 |
| | R | AAA AAG AAA GGA GGA ACA A | | |
| CYP2B1 | F | GCT CAA GTA CCC CCA TGT CG | 54 | 109 |
| | R | ATC AGT GTA TGG CAT TTT ACT GCG G | | |

**Hematological Measurements:**

Blood samples were collected from retro-orital of the experimental rats in capillary tubes coated with ethylene diamine tetra-acetic acid (EDTA). The tubes were immediately capped, kept at-4°C and were immediately analyzed for blood parameters using automated coagulating Sysmex apparatus of the type 8999. The hematological parameters included: hemoglobin (Hb) level (g/dl), red blood cells count (RBCs) and white blood cells count (WBCs) count in cells/cu.mm were analyzed according to methods of Dacice and Lewis [44] and Merghani [45].

**Histopathological study**

**Histological and histochemical studies:**

Specimens of all animals were dissected immediately after death, washed thoroughly with formal saline and then fixed in 10% neutral-buffered formal saline for 72 hours at least. All the specimens were washed in tap water for half an hour, dehydrated in ascending grades of alcohol (70% - 90% - 95% - absolute), cleared in xylene and then embedded in paraffin wax. Serial sections of 6 µm thick were cut and stained with

Haematoxylin and eosin [46] for histopathological investigation, Van Gieson's stain [46] for demonstration of collagen fibers.

All sections were investigated by the light microscope. Images were captured and processed using Adobe Photoshop version 8.0.

**DNA ploidy studies:**

Further sections were stained with Feulgen stain [47] for DNA studies and countered stained with Light green. DNA analysis was performed by Leica Quin 500 image cytometry in the Pathology Department, National Research Center. For each section (100-120) cells were randomly measured. The threshold values were defined by measuring control cells. The results are presented as histograms and tables which demonstrate the percentage of the diploid cells (2C), the triploid cells (3C), the tetraploid cells (4C) and the aneuploid cells (>5C). The DNA histogram is classified according to Danque *et al*., [48].

**Statistical Analysis:**

Data of DNA damage were analyzed using STATISTICA (Stat Soft, Tulsa, OK, USA) statistical package.

All data of gene expression were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [49] followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significance were based on probability of ($P \leq 0.05$).

Statistical analyses for biochemical parameters were performed by one way ANOVA followed by Tuckey's test or by two-way ANOVA followed by Bonferroni's test comparing all group. Analysis was conducted with Graph Pad Prism software V. 5.0.3 (Inc., San Diego, CA, USA). The significance of the differences among treatment groups was determined [50]. All statements of significance were based on probability of ($P \leq 0.05$).

## Results

**Genetic Results:**

**Comet Assay of DNA:**

The present results (Table, 2) showed that the proportion of DNA damage significantly increased in male rats treated with $CCL_4$ as compared to the control. This damage decreased by using moringa extract as a protective agent. The decreasing was increased by increasing the level of the dose. DNA damage was slightly reduced using 1.3 g/kg, while it significantly ($P \leq 0.05$) lowered using dose 2.0 g/kg and ($P \leq 0.001$) with 2.6 g/kg dose. Moreover, the highest dose 4.0 g/kg greatly ameliorated the genetic material, and gave the lowest proportion of DNA damage in respect to other lower doses of the moringa extract.

Best results (Table, 3) were obtained by using doses of moringa extract as a therapeutic agent in a dose dependent manner. Dose 1.3 g/kg insignificantly decreased the DNA damage given by $CCL_4$. Whereas, doses 2.0 or 2.6 g/kg significantly reduced the rates of such genetic abnormality. Moreover, dose 4.0 g/kg gave the lowest rate of DNA damge, where it caused significant reduction of DNA damage as compared to the other lower doses of moringa extract.

**Table (2): Effect of MOLE as a protective agent on DNA comet assay in $CCL_4$ intoxicated rats.**

| Treatment | No. of animals | DNA damage (comet assay) % |
|---|---|---|
| Negative control | 8 | $3.20 \pm 0.244^{a}$ |
| Control (olive oil) | 8 | $3.80 \pm 0.374^{a}$ |
| $CCL_4$ | 8 | $19.80 \pm 0.509^{e}$ |
| $CCL_4$ + 1.3 g | 8 | $19.0 \pm 0.547^{de}$ |
| $CCL_4$ + 2.0 g | 8 | $17.80 \pm 0.374^{d}$ |
| $CCL_4$ + 2.6 g | 8 | $15.60 \pm 0.748^{c}$ |
| $CCL_4$ + 4.0 g | 8 | $13.80 \pm 0.374^{b}$ |

**All data are expressed as mean ± SEM.**
**a,b,c,d,e means followed by different superscripts are significantly different (P≤0.05).**

**Table (3): Effect of MOLE as a therapeutic agent on DNA comet assay in $CCL_4$ intoxicated rats.**

| Treatment | No. of animals | DNA damage (comet assay) % |
|---|---|---|
| Negative control | 8 | $3.20 \pm 0.244^a$ |
| Control (olive oil) | 8 | $3.80 \pm 0.374^a$ |
| $CCL_4$ | 8 | $19.80 \pm 0.509^d$ |
| $CCL_4$ + 1.3 g | 8 | $18.80 \pm 0.509^d$ |
| $CCL_4$ + 2.0 g | 8 | $14.40 \pm 0.244^c$ |
| $CCL_4$ + 2.6 g | 8 | $6.80 \pm 0.489^b$ |
| $CCL_4$ + 4.0 g | 8 | $5.20 \pm 0.200^a$ |

**All data are expressed as mean ± SEM.**
**a,b,c,d means followed by different superscripts are significantly different (P≤0.05).**

**Gene Expression Analysis:**

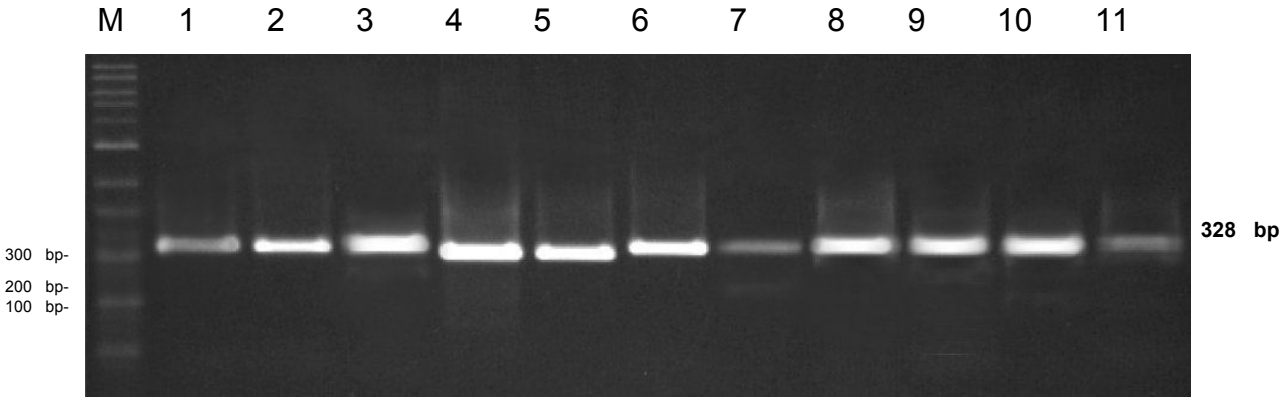
Semi-quantitative RT-PCR experiments were conducted to verify the expression of $P_{450}$ forms induced in rat liver as a result of $CCL_4$ treatment. CYP1A2 and CYP2B1 were selected as target genes in rat liver. The effect of MOLE on the expression of these genes was also examined. The gene transcripts (mRNAs) of the two genes CYP1A2 and CYP2B1 were successfully detected in all liver tissue within all treated groups (Fig. 1 and 2). The gene expression was normalized with the expression values of the $\beta$-Actin gene. The results (Fig. 1) revealed that CYP1A2 mRNA expression in the liver tissues of the group treated with $CCL_4$ was significantly higher ($P < 0.001$) than the control group. On the other hand, MOLE treatment was able to inhibit the up-regulation of the gene expression occurred by $CCL_4$. Moringa extract given along (as a protective agent) with $CCL_4$ slightly decreased the overexpression of CYP1A2 (given by $CCL_4$) in a dose dependent manner. But with the highest dose (4.0 g/kg) of MOLE, the mRNA expression of CYP1A2 gene was significantly down-regulated ($P \leq 0.05$) than those occurred in $CCL_4$ group.

Using MOLE as a therapeutic agent gave much better results. It down-regulated the overexpression of CYP1A2 gene (occurred by $CCL_4$) in a dose dependent manner (Fig. 1).

This down-regulation increased by increasing the dose level. By using the highest dose 4.0 g/kg the expression of CYP1A2 gene relatively regained its normal expression similar with those found in the control.

Also, the results (Fig. 2) revealed that CYP2B1 mRNA expression in the liver tissues was significantly higher ($P < 0.001$) in $CCL_4$ group in respect to normal control. This overexpression of CYP2B1 gene decreased by using MOLE treatment. Using MOLE as a protective agent slightly decreased the up-regulation of the gene expression occurred by $CCL_4$ in a dose dependent manner. This down-reuglation of the gene expression was nonsignificant using doses of 1.3 g/kg and 2.0 gm/kg., while it was significant with using doses of 2.6 gm/kg and 4.0 g/kg. Moreover, the highest dose 4.0 g/kg gave the lowest expression level of CYP2B1, but this expression level still higher significant than those found in the normal control.

On the other hand, best results were obtained by using doses of MOLE as a therapeutic agent in a dose dependent mannar. Dose 1.3 g/kg insignificantly decreased the overexpression given by $CCL_4$. Whereas, doses 2.0, 2.6 ($P \leq 0.05$) and 4.0 gm/kg ($P < 0.001$) significantly reduced the up-regulation of gene expression of CYP2B1. The dose 4.0 g/kg gave the lowest rate of such gene expression.



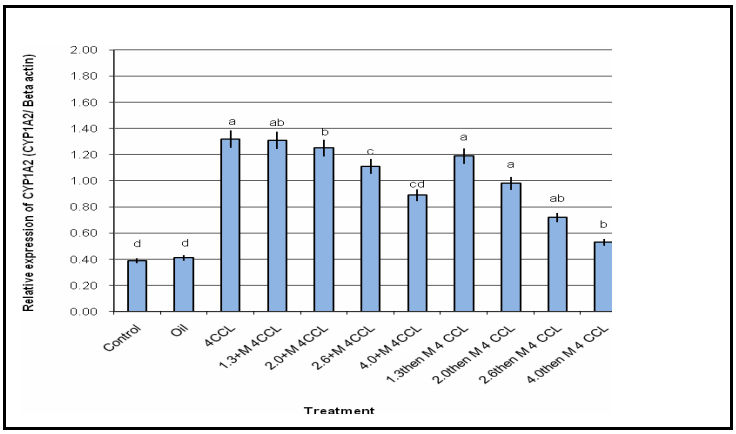
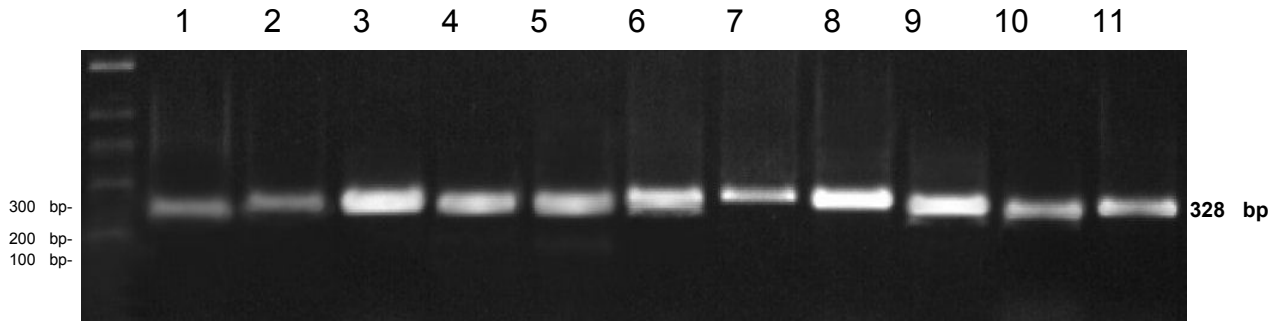



**Figure 1: Semi-quantitative RT-PCR confirmation of CYP1A2 gene in liver tissues of male rats treated with CCL4 combined with *M. oleifera.* M: DNA marker, Lane 1: control, Lane 2: oil solvent, Lane 3: CCL4, Lanes 4-7: CCL4+1.3, 2.0, 2.6 and 4.0 of *M. oleifera,* Lanes 8-11: CCL4 then 1.3, 2.0, 2.6 and 4.0 of *M. oleiferaI* for 3 weeks. Samples were normalized on the bases of β–actin expression.**
**a,b,c,d columns with different letters differ significantly (P ≤0.05).**



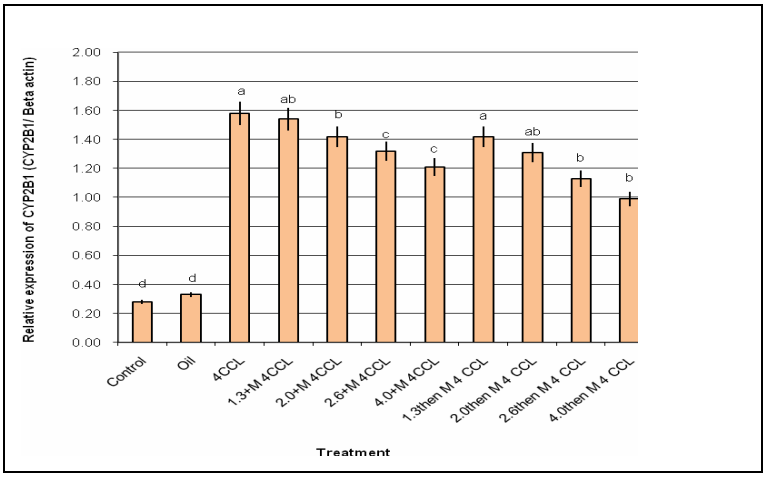




**Figure 2: Semi-quantitative RT-PCR confirmation of CYP2B1 gene in liver tissues of male rats treated with CCL4 combined with *M. oleifera.* M: DNA marker, Lane 1: control, Lane 2: oil solvent, Lane 3: CCL4, Lanes 4-7: CCL4+1.3, 2.0, 2.6 and 4.0 of *M. oleifera,* Lanes 8-11: CCL4 then 1.3, 2.0, 2.6 and 4.0 of *M. oleiferaI* for 3 weeks. Samples were normalized on the bases of β–actin expression.**
**a,b,c,d columns with different letters differ significantly (P ≤ 0.05).**

**Hematological parameters:**

The present results (Table, 4) revealed that Hb level significantly decreased (P≤0.001) in rats treated with $CCL_4$ as compared to those found in the control groups. In contrast, the Hb levels improved by using moringa extract as a protective agent. Dose 1.3 g/kg ameliorated the Hb level, however, this improvement was not significant. On the other hand, doses 2.0, 2.6 and 4.0 g/kg significantly enhanced the Hb level in respect to $CCL_4$ group. The dose 4.0 g/kg caused the highest level of Hb.

Also, $CCL_4$ treatment significantly reduced the RBCs count in comparison with control groups. Using moringa extract enhanced the RBCs cont. This enhancement was not significant with 1.3 g/kg dose, while it was significant with 2.0, 2.5 and 4.0 g/kg doses. The highest count of RBCs was obtained with 4.0 g/kg dose.

Moreover, the $CCL_4$ treatment induced the high significant increase in WBCs count in respect to the control groups. This damage was decreased using Moringa extraction as a protective agent in a dose dependent manner. Dose 1.3 g/kg slightly decreased such damage of WBCs. Whereas, doses 2.0, 2.6 and 4.0 g/kg significantly improved the WBCs count as compared to $CCL_4$ groups. The ability to induce the improvement of WBCs count was more effective by using 4.0 g/kg than other lower doses of moringa extract.

Best results of haematological examination were obtained using moringa extract as a therapeutic agent (Table, 5). Significant improvement of Hb level as well as RBCs and WBCs counts was induced with treatment of different doses of moringa extract. This improvement was increased by increasing the dose level. The highest dose 4.0 g/kg greatly ameliorated the hematological parameters and caused relatively similar results as those found in the control.

**Table (3): Effect of MOLE as a protective agent on hematological parameters in $CCL_4$ intoxicated rats.**

| Treatment | Hematological parameters | | |
|---|---|---|---|
| | Hb (g/dl) | RBCs (cumm) | WBCs |
| Control | $17.60\pm0.509^{d}$ | $7.80\pm0.200^{e}$ | $7.80\pm0.374^{a}$ |
| Control oil | $16.80\pm0.374^{d}$ | $6.40\pm0.244^{d}$ | $7.60\pm0.600^{a}$ |
| $CCL_4$ | $7.20\pm0.374^{a}$ | $2.20\pm0.200^{a}$ | $23.80\pm0.374^{e}$ |
| $CCL_4$ + 1.3M | $9.00\pm0.447^{ab}$ | $2.80\pm0.200^{a}$ | $23.20\pm0.200^{de}$ |
| $CCL_4$ + 2.0M | $10.60\pm1.661^{bc}$ | $3.60\pm0.244^{b}$ | $22.20\pm0.374^{cd}$ |
| $CCL_4$ + 2.6M | $11.80\pm0.374^{c}$ | $4.20\pm0.200^{bc}$ | $21.20\pm0.374^{c}$ |
| $CCL_4$ + 4.0M | $12.80\pm0.374^{c}$ | $4.60\pm0.400^{c}$ | $19.20\pm0.374^{b}$ |

**All data are expressed as mean ± SEM.**
**a,b,c,d,e means followed by different superscripts are significantly different (P≤0.05).**

**Table (4): Effect of MOLE as a a therapeutic agent on hematological parameters in $CCL_4$ intoxicated rats.**

| Treatment | Hematological parameters | | |
|---|---|---|---|
| | Hb (g/dl) | RBCs (cumm) | WBCs |
| Control | $17.60\pm0.509^{e}$ | $7.80\pm0.200^{e}$ | $7.80\pm0.374^{a}$ |
| Control oil | $16.80\pm0.374^{de}$ | $6.40\pm0.244^{cd}$ | $7.60\pm0.600^{a}$ |
| $CCL_4$ | $7.20\pm0.374^{a}$ | $2.20\pm0.200^{a}$ | $23.80\pm0.374^{f}$ |
| $CCL_4$ + 1.3M | $10.20\pm0.489^{b}$ | $3.80\pm0.374^{b}$ | $20.20\pm0.200^{e}$ |
| $CCL_4$ + 2.0M | $12.40\pm1.435^{c}$ | $4.40\pm0.244^{b}$ | $16.00\pm0.447^{d}$ |
| $CCL_4$ + 2.6M | $15.20\pm0.489^{d}$ | $5.60\pm0.600^{c}$ | $13.20\pm0.374^{c}$ |
| $CCL_4$ + 4.0M | $16.60\pm0.244^{d}$ | $6.80\pm0.374^{d}$ | $9.20\pm0.374^{b}$ |

**All data are expressed as mean ± SEM.**
**a,b,c,d,e,f means followed by different superscripts are significantly different (P≤0.05).**

**Histopathological results:**

The results of this study revealed that $CCl_4$ caused massive damage to liver tissue in the form of excessive fibrosis, cellular infiltration and vacuolar degeneration of hepatocytes (Fig. 3, c & d). This damaging effect was slightly reduced by using moringa extract as a protective agent (given with $CCl_4$) where fibrosis and vacuolar degeneration were slightly decreased with dose 2.0 g/kg (Fig. 4, B). This reduction was increased with dose 2.6 g/kg (Fig. 4, C), while dose 4.0 g/kg preserved the normal structure of the liver tissue in spite of the presence of focal aggregations of inflammatory cells (Fig. 4, D).

Better results were obtained by using moringa extract as a therapeutic agent. These results were dose dependent as the lowest dose 1.3 g/kg didn't reduce fibrosis or vacuolar degeneration obviously (Fig. 5, A), dose 2.0 g/kg markedly reduced fibrosis and degeneration (fig. 5,B), while dose 2.6 g/kg greatly ameliorated the liver tissue except for mild cellular infiltration around main blood vessels (Fig. 5, C). The best results were

obtained by using dose 4.0 g/kg as the liver tissue retained its normal structure and became close to normal (Fig. 5, D).

**Histochemical results:**

Using Van Gieson's stain (stains collagen fibers red) confirmed the histopathological results as they revealed that normal liver tissue contains very little amount of collagen fibers concentrated around the central vein and in portal area (Fig. 6, A). $CCl_4$ increased the collagenous fibers greatly in the liver tissue (Fig. 6, B). Moringa extract given along with $CCl_4$ decreased slightly collagen fibers in a dose dependent manner (Fig. 6, C, D, E), but even with the highest dose some collagen fibers were still observed in the liver tissue (Fig. 6, F).

Using moringa extract as a therapeutic agent gave much better results. It reduced fibrosis in a dose dependent manner (Fig. 7, C, D & E). By using the highest dose, the liver tissue regained its normal structure (Fig. 7, F).

Liver tissue sections from rats received vehicle only were close to normal in both histopathological (Fig. 3, B) and histochemical (Fig, 7, A) results.

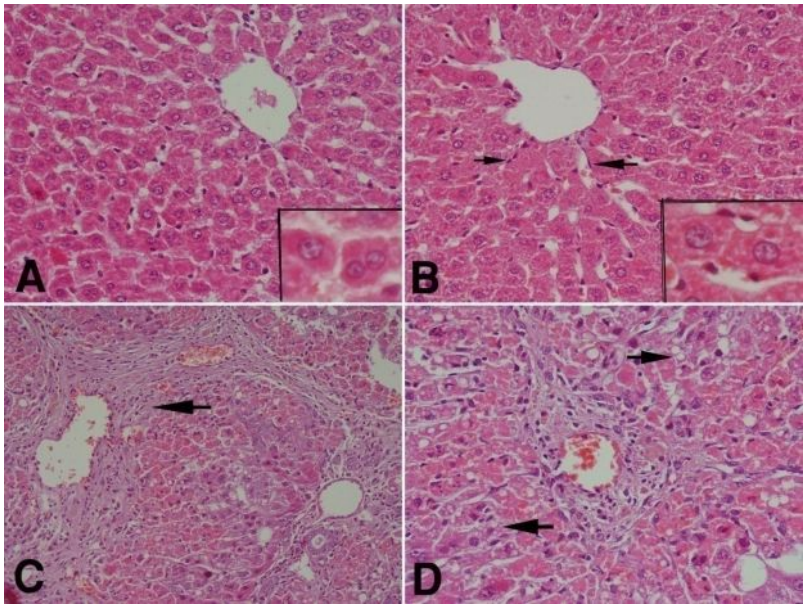



**Fig. 3: A photomicrograph of liver tissue of a rat (a) shows the normal structure of liver tissue and the right lower corner part of the figure shows the normal shape of hepatocytes. (b) a section of liver tissue from a rat received vehicle only, that appears close to normal. Notice Kuppffer cells with flattened nuclei in blood sinusoids (arrow). (C) a section of liver tissue from a rat received $CCl_4$, showing massive fibrosis specially around dilated central veins (arrow) that extend to form continuous wide band surrounding the lobules. The normal architecture of the lobules is greatly distorted. (D) another section of the liver tissue from the same group showing fibrosis with cellular infiltration around central vein and variable degrees of vacuolar degeneration in many hepatocytes (arrow).**
**(Hx. &E. X 400 for A, B & D, X 200 for C & X1000 for the lower right corners)**

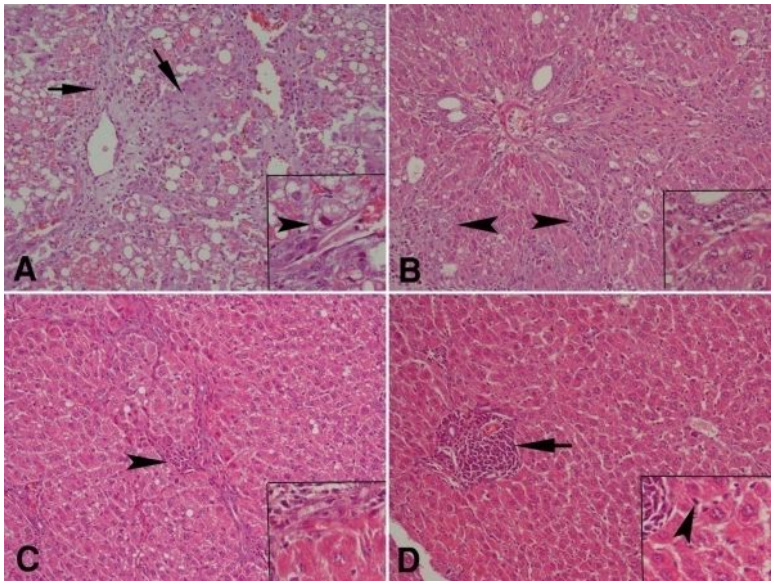



**Fig. 4: A photomicrograph of sections of liver tissue (A) from a rat received 1.3 g/kg moringa with $CCl_4$, shows no improvement as massive fibrosis (arrow) and severe vacuolar degeneration (arrowhead in the lower right corner) are still present. (B) from a rat received 2.0 g/kg moringa with $CCl_4$, shows minimal improvement in the form of slight reduction of fibrosis and dilatation of blood vessels. However, focal aggregations of cellular infiltrates (arrowhead) are noticed. The lower right corner of the figure shows mild reduction of vacuolar degeneration. (C) from a rat that received 2.6 g/kg moringa with $CCl_4$, shows noticeable amelioration as there is marked reduction of fibrosis and cellular infiltration (arrowhead), although the liver tissue architecture is still deformed. (D) from a rat that received 4.0 g/kg moringa with $CCl_4$, shows great amelioration of tissue, where there is retrieval of the normal architecture of liver tissue, although there is solitary focal aggregation of inflammatory cells (arrow) and mild increase in Kuppffer cells (arrowhead in the lower right corner of the figure).**
**(Hx. &E. X 200 & X 400 for the lower right corners)**

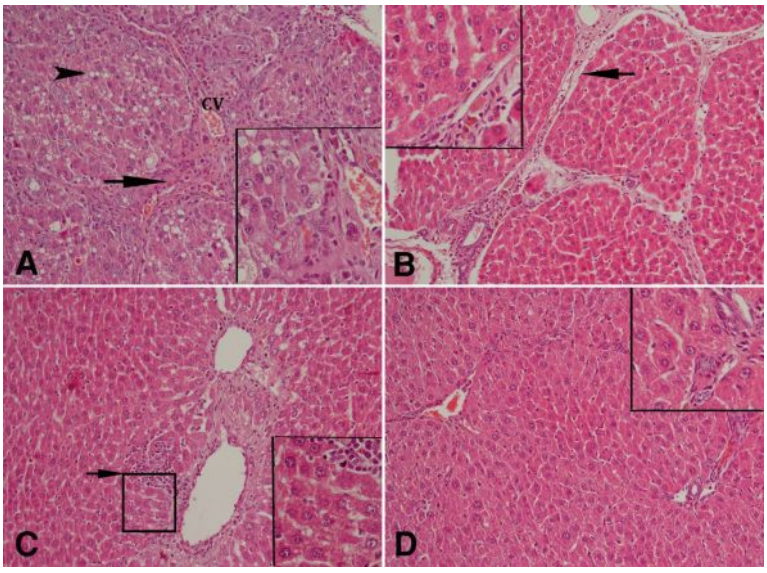



**Fig. 5: : A photomicrograph of sections of liver tissue (A) from a rat received $CCl_4$ and then 1.3 g/kg moringa, shows complete deformation of the general architecture of the tissue, extensive fibrosis with cellular infiltration (arrow) specially around dilated and congested blood vessels, massive vacuolar degeneration (arrowhead), that appears more clearly in the right lower corner of the figure. (B) from a rat received $CCl_4$ and then 2.0 g/kg moringa, shows noticeable improvement in the form of noticeable reduction of fibrosis and cellular infiltration in between hepatocytes (arrow). The upper left corner of the figure shows healthy and normally-shaped hepatocytes. (C) from a rat received $CCl_4$ and then 2.6 g/kg moringa, it shows marked amelioration as the tissue regained its normal architecture, while fibrosis and cellular infiltration are restricted to the area around blood vessels (arrow). (D) from a rat received $CCl_4$**

**and then 4.0 g/kg moringa, shows hepatic tissue that is very close to normal except for very fine bands of fibrous tissue at the portal area and around central vein.**
**(Hx. &E.X 200 & X 400 for the lower right corners)**

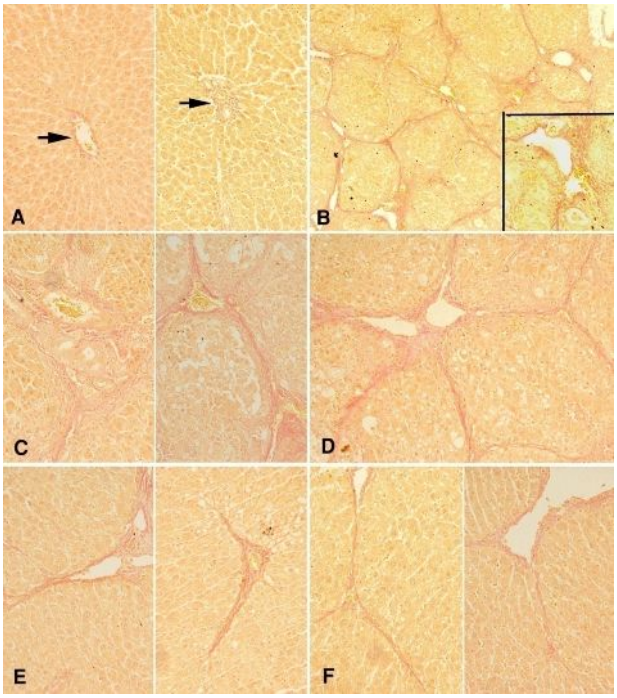



**Fig. 6: photomicrograph of sections of liver tissue from (A) control rat that shows only very few connective tissue fibers (red colored) around central vein and in portal area (arrows). (B) a rat received $CCl_4$ shows extensive connective tissue fibers all over the tissue surrounding the lobules and blood vessels. (C) a rat received 1.3 g/kg moringa as a protective agent shows that extensive connective tissue fibers are still present. (D) a rat received 2.0 g/kg moringa that shows slight reduction of fibrous tissue as compared with the previous section. (E) a rat received 2.6 g/kg moringa shows noticeable reduction of fibrous tissue. (F) a rat received 4.0 g/kg moringa where a marked amelioration is observed, where a small amount of fibrous tissue appears around blood vessels extending as thin bands between hepatocytes.**

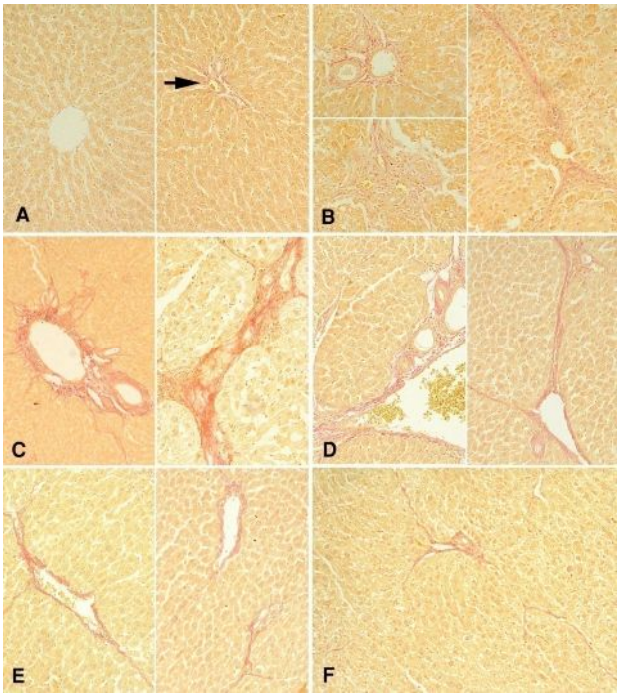
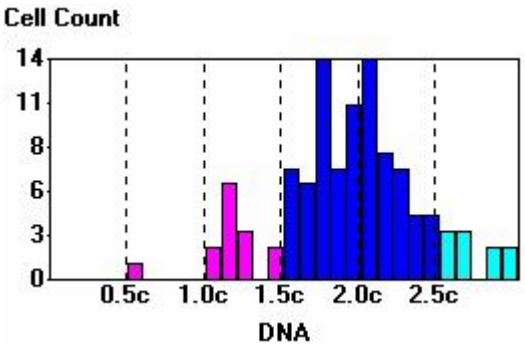


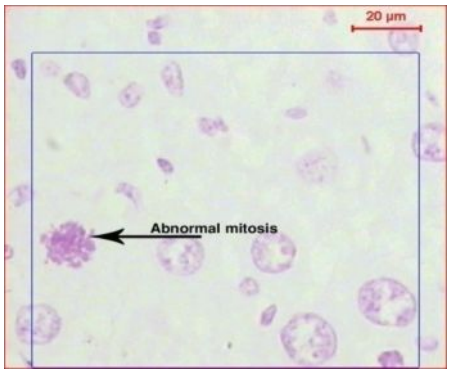

**Fig. 7: photomicrograph of sections of liver tissue from (A) a rat received olive oil only (vehicle) shows only very few connective tissue fibers (red colored) in portal area (arrows). (B) a rat received $CCl_4$ shows extensive connective tissue fibers all over the tissue surrounding the lobules and blood vessels. (C) a rat**

**received $CCl_4$ and then moringa extract in a dose (1.3 g/kg) shows that collagenous fibers (in red) are still excessive in portal area and in between hepatocytes. (D) a rat received $CCl_4$ and then moringa extract (2.0 g/kg) shows slight reduction in collagen fibers. (E) a rat received $CCl_4$ and then moringa extract (2.6 g/kg) shows marked reduction in collagenous fibers being restricted only around central veins. (F) a rat received $CCl_4$ and then moringa extract (4.0 g/kg) shows normalization of liver tissue.**

**DNA ploidy results:**



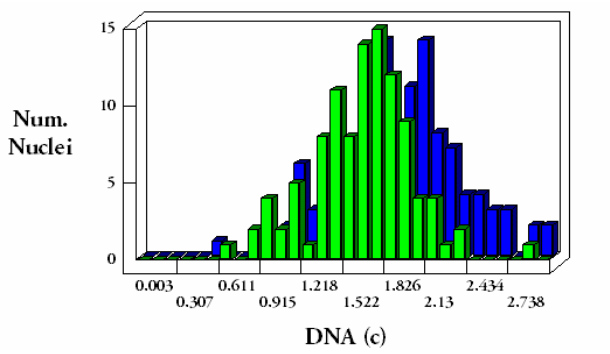

**Histogram (1) C –ve**



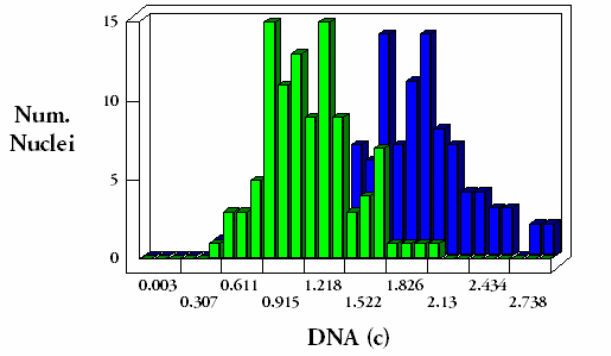

**Feulgen X 400**

**Table (6) C –ve**

| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 106 | 100.0% | 1.000 | 0.208 | 0.144 | 1.957 | 2.105 | 0.457 | 23.322 | 0.553 | 3.284 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 14 | 13.208% | 0.603 | 0.716 | 0.412 | 1.180 | 1.112 | 0.217 | 18.430 | 0.553 | 1.480 |
| 1.5c-2.5c | 80 | 75.472% | 1.009 | 0.059 | 0.044 | 1.975 | 2.114 | 0.243 | 12.299 | 1.530 | 2.455 |
| 2.5c-3.5c | 12 | 11.321% | 1.404 | 0.611 | 0.364 | 2.748 | 2.560 | 0.236 | 8.594 | 2.503 | 3.284 |
| 3.5c-4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| > 4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |



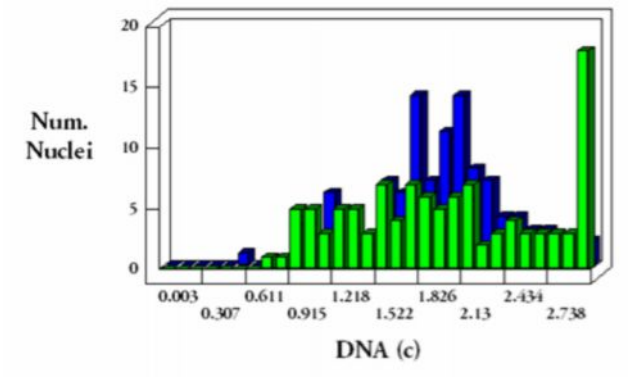

**Histogram (2) Cont. Vehicle**



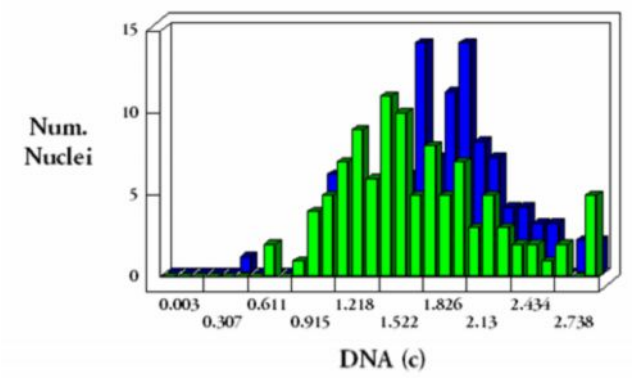

**Histogram (3) C +ve ($CCl_4$)**

**Table (7) Cont. Vehicle**

| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 104 | 100.0% | 0.847 | 0.247 | 0.168 | 1.657 | 1.742 | 0.361 | 21.807 | 0.678 | 2.895 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 34 | 32.692% | 0.640 | 0.605 | 0.361 | 1.253 | 1.432 | 0.221 | 17.651 | 0.678 | 1.483 |
| 1.5c-2.5c | 69 | 66.346% | 0.939 | 0.062 | 0.046 | 1.839 | 1.777 | 0.192 | 10.419 | 1.524 | 2.359 |
| 2.5c-3.5c | 1 | 0.962% | 1.479 | 0.800 | 0.449 | 2.895 | 2.224 | - | - | 2.895 | 2.895 |
| 3.5c-4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| > 4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |




**Histogram (4)**




**Histogram (5)**

**Table (8) C +ve ($CCl_4$)**

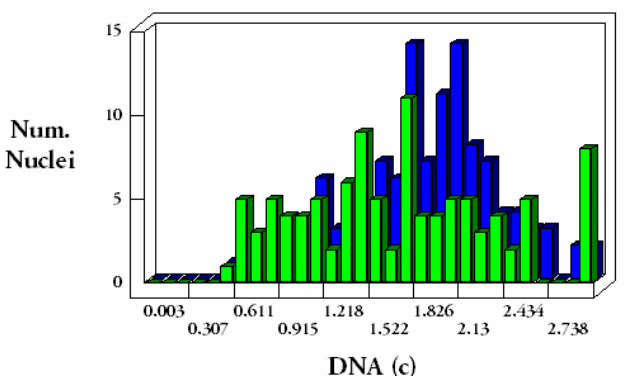
| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 102 | 100.0% | 0.637 | 0.672 | 0.392 | 1.248 | 1.139 | 0.327 | 26.211 | 0.551 | 2.203 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 82 | 80.392% | 0.576 | 0.813 | 0.454 | 1.127 | 1.129 | 0.225 | 20.006 | 0.551 | 1.496 |
| 1.5c-2.5c | 20 | 19.608% | 0.891 | 0.094 | 0.068 | 1.744 | 1.745 | 0.172 | 9.873 | 1.510 | 2.203 |
| 2.5c-3.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| 3.5c-4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| > 4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |

**Table (9)**

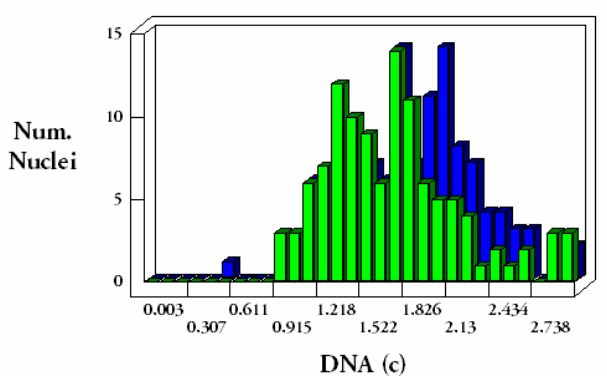
| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 109 | 100.0% | 1.088 | 0.801 | 0.449 | 2.129 | 2.107 | 0.890 | 41.777 | 0.748 | 5.322 |
| 5cER | 1 | 0.917% | 2.719 | 11.034 | 1.898 | 5.322 | 2.661 | - | - | 5.322 | 5.322 |
| < 1.5c | 27 | 24.771% | 0.589 | 0.753 | 0.428 | 1.153 | 1.418 | 0.190 | 16.483 | 0.748 | 1.428 |
| 1.5c-2.5c | 51 | 46.789% | 0.998 | 0.078 | 0.057 | 1.953 | 2.173 | 0.278 | 14.251 | 1.503 | 2.497 |
| 2.5c-3.5c | 22 | 20.183% | 1.497 | 0.941 | 0.506 | 2.931 | 2.840 | 0.281 | 9.575 | 2.530 | 3.434 |
| 3.5c-4.5c | 7 | 6.422% | 1.962 | 3.428 | 1.135 | 3.840 | 3.882 | 0.219 | 5.703 | 3.551 | 4.200 |
| > 4.5c | 2 | 1.835% | 2.558 | 9.145 | 1.768 | 5.008 | 5.322 | 0.444 | 8.866 | 4.694 | 5.322 |

**Table (10)**

| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 103 | 100.0% | 0.932 | 0.457 | 0.287 | 1.824 | 1.590 | 0.656 | 35.971 | 0.787 | 4.564 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 33 | 32.039% | 0.634 | 0.609 | 0.363 | 1.241 | 1.393 | 0.182 | 14.690 | 0.787 | 1.498 |
| 1.5c-2.5c | 60 | 58.252% | 0.969 | 0.088 | 0.064 | 1.898 | 1.614 | 0.280 | 14.773 | 1.514 | 2.477 |
| 2.5c-3.5c | 6 | 5.825% | 1.427 | 0.701 | 0.406 | 2.794 | 2.655 | 0.292 | 10.457 | 2.567 | 3.361 |
| 3.5c-4.5c | 3 | 2.913% | 1.998 | 3.656 | 1.174 | 3.911 | 3.962 | 0.097 | 2.491 | 3.807 | 4.000 |
| > 4.5c | 1 | 0.971% | 2.332 | 6.576 | 1.545 | 4.564 | 4.245 | - | - | 4.564 | 4.564 |




**Histogram (6)**




**Histogram (7)**

**Table (11)**

| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 102 | 100.0% | 0.884 | 0.579 | 0.349 | 1.731 | 1.796 | 0.715 | 41.333 | 0.598 | 3.819 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 39 | 38.235% | 0.536 | 0.982 | 0.522 | 1.049 | 0.623 | 0.282 | 26.889 | 0.598 | 1.486 |
| 1.5c-2.5c | 49 | 48.039% | 0.976 | 0.088 | 0.064 | 1.910 | 1.767 | 0.285 | 14.943 | 1.505 | 2.440 |
| 2.5c-3.5c | 12 | 11.765% | 1.476 | 0.893 | 0.487 | 2.890 | 2.613 | 0.332 | 11.485 | 2.518 | 3.458 |
| 3.5c-4.5c | 2 | 1.961% | 1.887 | 2.885 | 1.036 | 3.694 | 3.819 | 0.176 | 4.774 | 3.569 | 3.819 |
| > 4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |

**Table (12)**

| Range | Tot Cells | % Cells | DNA Index | 2cDI | DNA MG | Mean | Mode | Std. Dev. | CV | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| All | 113 | 100.0% | 0.899 | 0.290 | 0.194 | 1.760 | 1.766 | 0.484 | 27.503 | 0.946 | 3.415 |
| 5cER | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| < 1.5c | 39 | 34.513% | 0.662 | 0.519 | 0.319 | 1.295 | 1.472 | 0.150 | 11.608 | 0.946 | 1.500 |
| 1.5c-2.5c | 64 | 56.637% | 0.956 | 0.071 | 0.052 | 1.871 | 1.757 | 0.235 | 12.545 | 1.508 | 2.436 |
| 2.5c-3.5c | 10 | 8.85% | 1.460 | 0.800 | 0.448 | 2.858 | 2.918 | 0.264 | 9.253 | 2.513 | 3.415 |
| 3.5c-4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |
| > 4.5c | 0 | 0.0% | - | - | - | - | - | - | - | - | - |

DNA content in all the studied groups: In the present study, the Qwine 500 image analyzer was used to evaluate the DNA content. The image analysis system automatically express the DNA content of each individual cell measured then gave the percentage of each cell out of the total number of cells examined. Also, it classifies the cells into four groups; diploid (2C), proliferating cells (3C), tetraploid (4C) and aneuploid cells (>5C). The proliferating cells were further classified into; (<10%) low proliferation index, (10-20%) medium proliferation index and (>20%) high proliferation index.

Normal distribution of DNA content in the liver cells of the control group showed that 13.2 % of the examined cells contained DNA (<1.5C), 75.47% contained diploid DNA value (2C), 11.32% contained (3C) DNA value (medium Proliferation Index) and 0.0% of the examined cells at (4C) area (Histogram 1& Table 6).

Examination of cells from group of rats treated with olive oil only (vehicle) showed that 32.69% of cells contained DNA value (< 1.5 C), 66.34% of examined cells contained (2C) DNA value and only 0.96% of cells contained (3C) DNA value, while 0.0% of cells were in the (4C) DNA value (Histogram 2 & Table 7). These results are comparable with the control group

Examination of cells from the +ve control group treated with $CCl_4$ (Histogram 3 & Table 8) showed that the cells contained DNA (<1.5C) were 80.39%, while 19.6% contained DNA value (2C), which means decrease in DNA content (hypoploidy) compared to the control. 0.0% of examined cells contained DNA value (3C) and (4C).

Examination of cells from the group treated with moringa as a protective agent (dose 2.0 g/kg) and $CCl_4$ show that cells contained DNA (<1.5C) were 24.77%, cells contained DNA value (2C) were 46.78%, while cells contained (3C) DNA value were 20.18% (high proliferation index) and 6.42% of cells were in (4C) area ( Histogram 4 & Table 9). 32.03% of the examined cells from the group treated with dose 4.0 g/kg of moringa and $CCl_4$ contained DNA value (<1.5 C), 58.25% of cells contained DNA value (2C), while 5.82% of the examined cells contained (3C) DNA value (low proliferating index) and 2.91% of the examined cells contained (4C) DNA value (Histogram 5 & Table 10). These results revealed amelioration of the DNA content of examined cells that was dependent with moringa dose.

Examination of cells from the group treated with the dose 2.0 g/kg of moringa as a therapeutic agent after $CCl_4$ treatment showed that 38.23% of cells contained (<1.5C) DNA value, 48.03% of cells contained DNA value (2C), 11.76% of cells contained (3C) DNA value (medium proliferating index) and only 1.96% of cells were in the (4C) area (Histogram 6 & Table 11).

Examination of cells from the group of rats received moringa in a dose of 4.0 g/kg after treatment with $CCl_4$ showed that 34.51% of cells contained DNA value (< 1.5 C), 56.63% of cells contained (2C) DNA value, while 8.85% 0f cells contained (3C) DNA value (low proliferating index) and 0.0% of cells were in (4C) area (Histogram 7 & Table 12).

These results indicate that treatment with moringa along with $CCl_4$ showed DNA values better than those of group of animals treated with $CCl_4$ alone. Treatment with moringa after $CCL_4$ treatment showed DNA values comparable to the control values specially with high dose of extract, while group treated with $CCl_4$ showed decreased DNA values (hypoploidy).

## Discussion

### Genetic Studies:

The present results revealed that treatment of male rats with $CCL_4$ significantly increased the DNA damage in blood cells and the significant elevated the expression of CYP1A2 and CYP2B1 genes in liver tissues compaed with the control group. This study proved that $CCL_4$ treatment has a mutagenic effect on genomic material of male rats. Our findings on DNA damage are in agreement with that reported by Abdou *et al*. [35, 36] who found that administrations of $CCL_4$ to rats caused significant increase of DNA damage as compaed to normal control. The DNA damage can originate from the direct modification of DNA by chemical agents or their metabolites; from the processes of DNA excision repair, replication and recombination; or from the process of apoptosis [51].

Following administration, $CCL_4$ is activated by cytochrome $P_{450}$ system to form trichloromethyl ($CCL_3$) radical. This radical binds to cellular molecules (nucleic acids, proteins and lipids) thereby impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration, while the reaction between trichloromethyl ($CCL_3$) radical and DNA is thought to function as initiator of heaptic cancer [52]. This radical can also react with oxygen to form the trichloromethylpheroxy ($CCL_3OO$) radical, a highly reactive species. This compound initiates the chain reaction of lipid peroxidation, culminating in destruction of polyunsaturated fatty acids, especially those associated with phospholipids [52]. This leads to alteration of permeabilities of mitochondrial, endoplasmic reticulum and plasma membranes, resulting in the loss of cellular calcium sequestration and disruption of calcium, homeostasis with subsequent cell damage [53-55]. Oxidative stress induced by oxygen-derived species can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA protein, cross-links and base-free sites. If left un-repaired, oxidative DNA damage can lead to detrimental biological consequences in organisms, including cell death, mutations and

transformation of cells to malignant cells [36]. Previous studies proved that changes in genomic DNA could reflect DNA alterations from single base changes (point mutations) to complex chromosomal rearrangements [56, 57].

So, the DNA damage formation causes genomic instability including gene expression of animal genes [58-62].

Regarding the gene expression alterations, our results indicated that CYP1A2 mRNA and CYP2B1mRNA expression in liver tissues of $CCL_4$ group was significantly higher than the normal control. Similarly, $CCL_4$ has also been reported to activate and increase the expression of tumor necrosis factor (TNF)α, nitric oxide (NO) and transforming growth factors (TGF)-α and-β in the cell, processes that appear to direct the cell primarily toward self-destruction or fibrosis. TNF-α pushes the cell toward apoptosis [63], whereas, the TGFs appear to direct toward fibrosis [64, 55].

Moreover, in this study the over expression of cytochrome $P_{450}$ genes induced by $CCL_4$ of CYP1A2 and CYP2B1 mRNA in rat liver is similar to that reported in another studies for phenobarbital and propiconazole. In rats, phenobarbital increased hepatic CYP2B1 as measured either by mRNA content or wester analyses [65, 66]. In mice phenobarbital increased hepatic CYP1A2 mRNA [67]. Sun *et al*. [68] studied the effect of propiconazole as a fungicide on the expression of heaptic cytochrome $P_{450}$ genes in male rats and mice. Quantitative real time RT-PCR assays of rat hepatic RNA samples from treated animals with the propiconazole revealed significant mRNA overexpression of CYP1A2 and CYP2B1 genes as compard to normal control. Similarly, Eshak *et al*. [69] found over expression level of CYP1A2 gene in mice fed diet contaminated with industrial wastewater-phenols in respect to those found in mice fed basal normal diet.

CYPO1A2 is constitutively expressed in the liver of mice, rats and humans and is also inducible in these mammalian species by structurally diverse compounds. CYP1A2 gene expression is regulated by AhR receptor [70, 71] and the pregnane X receptor (PXR) [72]. CYP1A2 has been found to metabolize drugs and the N-hydroxylate aromatic amines [73] (Kobayashi *et al*., 2002). CYP2B1 is constitutively expressed in mouse, rat and human livers and is inducible in these species by drugs and related chemicals [74, 66]. Hepatic CYP2B1 has been shown to have catalytic activity in the metabolism of lipophilic drugs and environmental chemicals and the hydroxylation of steroids [75]. CYP2B1 gene expression is regulated by the constitutive androstan receptor (CAR) as well as PXR in mouse and human livers [72] and by CAR in rat liver [74]. As discussed above, $CCL_4$ seems to act like phenobarbital and propiconazole in that they are members of a class of chemicals that presumably activate both PXR and CAR.

**Effect of MOLE on genetic alterations:**

The present results revealed that MOLE treatments significantly reduced DNA damage and significantly down-regulated the expression of CYP genes (CYP1A2 and CYP2B1) compared to $CCL_4$ group. These findings proved that MOLE has a protective and therapeutic role in body cells against the observed mutagenic effect of $CCL_4$. The amelioration role of MOLE may be attributed to the potential involvement of phytomolecules (natural antioxidants) of the extract to interfere with cytochrome enzymes participating in biotransformation of $CCL_4$ to genotoxic metabolites [28].

Prasanna and Sreelatha [76] stated that MOLE treatment can act as effective modulators in reducing the toxicity in cells under oxidative stress by enhancing the stimulation of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) that are capable of removing oxygen radicals and their products and/or repairing the damage caused by oxidation stress. On the other hand the free radical scavenging represents one of the important strategies in antimutagenesis and anticarcinogenesis [77, 78]. MOLE contains a rich amount of antoxidants [79, 80]. A possible explanation for the protective or therapeutic effect revealed in the present investigation of its antoxidant and scavenging properties. Antioxdiants provide protection or remediation by scavenging reactive oxidative species (ROS) that damage DNA and initate diseases such as cancer [28].

MOLE is an excellent source of antioxidant constituent such vitamin C [4]. It has been reported that supplementation of the diet with vitamin C results in a highly significant decrease in endogenous oxidative base damage in the DNA of patients lymphocytes and hymphocytes of antoxidant-supplemented subjects showed increase resistance to oxidative damage *in vitro* [81]. Also vitamin C was known to be immune system booster and reduces the free radical in the body [36].

Furthermore, dietary antioxidants protect against oxidative damage to DNA, proteins and lipids and have a significant impact on the regulation of gene expression [82 ,76].

Scientists noted that MOLE contains a potent antioxidant of polyphenols [4] ,83]. This component was known to inhibit gene expression in androgen-independent protest cancer cells. It suppresses the growth of cancer cells by interfering with its genetic factors [35]. Also, polyphenols can inhibit a specific protein found in bone marrow and which is responsible for cancer in bone and increased the production of antioxidants in the sperms [35]. The antioxidative properties of MOLE polyphenols are thought to arise from their reactivity as hydrogen or electron donors from their ability to stabilize unpaired electrons and to terminate fenton reactions [35]. High content of polyphenols and alkaloids in MOLE [28] 4] might act as free radical scavengers by preventing and repairing damages caused by oxidative stress through the generation of ROS and therefore could enhance the immune defense and lower the risk of cancer and degenerative diseases [76].

Moreover, these antioxidant constituents of MOLE can inhibit cancer mutation by latching onto DNA-masking sensitive sites on the genetic material that might be occupied by harmful chemicals [84, 85, 35].

MOLE contains a compound called niacimicin that was shown to be a potent inhibitor of cancer [86, 85]. Also, this compound inhibited tumor promotion in a mouse two-stage DMBA-TPA tumor model [16].

**Hematological Study:**

The present results showed that the treatment with $CCL_4$ to rats significantly decreased the RBC count and Hb level, however, the results observed significant increase ($P < 0.05$) in the level of WBC as compared to normal control. Our findings are in agreement with that reported by Saba *et al*. [55], Sule *et al*. [87] and Elshater *et al*. [88], who found that the administration of $CCL_4$ to rats led to significant decrease of RBC counts and Hb level and significant increase of WBC counts in respect to normal control. Also, the present results were similar to that reported by Mada *et al*. [99], who indicated to a significant decrease ($P < 0.05$) in the level of Hb and packed cell volume (PCV) in rats treated with $CCL_4$ when compared with normal control.

The depression in RBCs count and Hb content might be attributed to the toxicity of $CCL_4$ that has been known to produce hepatic damage by generation of highly reactive trichloromethyl ($CCL_3$) and trichloromethylperoxy ($CCL_3OO$) radical when metabolized by cytoxhrome $P_{450}$ [89, 54]. This toxicity lead to transient decrease in the Hb concentration and reticulocyte count as well as PCV and RBCs counts by extension [90, 55]. Also this agrees with earlier report by Jain [91], who observed that xenobiotics can cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane is oxidized which infects injury to the erythrocyte membrane. Moreover, Essawy *et al*. [92] and Elshater *et al*. [88] revealed that the depression in RBCs count and Hb level due to $CCL_4$ treatment could be attributed to disturbed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and/or their enhanced removal circulation.

Furthermore, xenobiotic oxidants cause elevations in lipid peroxides in red cells [93] (Maduka and Okoye, 2002) and accompanying reductions in physiological parameters such as red cell mutaration factors, RBCs and Hb [94-96].

On the other hand, the $CCL_4$ treatment increased WBCs count. This may be attributed to the defensive mechanism of immune system [97], so the ability of free radical to increase WBCs count indicates that these radicals to an extent affected the defense mechanism of treated rats [98, 88].

**Effect of MOLE on hematological parameters:**

In the present study, the treatment with MOLE was observed to significantly ameliorated the hematological parameters and stimulated them towards normal value especially in high dose of (4.0 g/kg.b.w.) the extract. These ameliorations of hematological parameters by MOLE suggests cytoprotection and therapeutic effect by the presence of antioxidant properties against $CCL_4$. This might be due to the high content of ascorbic acid in MOLE [17, 14, 18, 4]. This constituent plays an important role in iron absorption and its transport. So, it supplies iron for development and maturation of RBCs [88], which in turn increases the level of Hb and PCV.

Also, the abnormal parameters of hematology caused by $CCL_4$ were minimized by MOLE treatment, as flavonoids one of its active components [18, 4] are known to be vasculo-protector and powerful antioxidant [87] as well as the flavonoids probability did so by reducing the accumulation of toxic $CCL_4$ derived metabolites [99]. Blood parameters were found to be positively affected by using *M. oleifera* as a therapeutic

herb [100] through enhancing the activities of antioxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals [101, 18]. Osman *et al*., [102] showed that the rats which administrated with MOLE had significant increases in RBCs, PCV and Hb level as compared to rats fed basic diet alone.

Moreover the amelioration of hematological parameters that observed in the present study might be due to the presence of higher rates of free radical scavengers such as vitamin A,B,C and E, β-carotene and various phenolic in MOLE [15, 14], these components have been reported in other studies to reduce the toxic effects of various toxicants including $CCL_4$ [28, 55, 87, 88, 96, 99].

**Histopathological and histochemical studies:**

Histopathological and histochemical results of the present studies revealed that $CCl_4$ caused massive fibrosis of liver tissue that was partially prevented and markedly reduced by using moringa extract as a protective or therapeutic agent respectively. Histopathological results of this study clarified that $CCl_4$ had a severe damaging effect on hepatocytes in the form of vacuolar degeneration. Using moringa extract along with $CCl_4$ reduced markedly this effect especially with high doses, which might be due to its antioxidant components recorded by Bertling *et al*. [103] as follows: Total anti-oxidant (TAO) (1.8 mg /sup), leaf-ascorbic acid (AsA) (2.0 mg /sup), and total phenols (TP) (64.1 micro g /sup).

Such results are coinciding and can be explained by those of [104] who stated that moringa extract reduced liver damage as well as symptoms of liver fibrosis, also, the immunohistochemical study showed that moringa extract markedly reduced the numbers of smooth muscle alpha-actin-positive cells and the accumulation of collagens I and III in liver. Moringa extract showed significant inhibitory effect on 1,1-diphenyl-2-picrylhydrazyl free radical, as well as strong reducing antioxidant power. These results suggested that Moringa extract can act against CCl4-induced liver injury and fibrosis in rats by a mechanism related to its antioxidant properties, anti-inflammatory effect and its ability to attenuate the hepatic stellate cells activation.

Chattopadhyay *et al*. [105], reported that Administration of aqueous extract of *M. oleifera* was found to significantly prevent the arsenic-induced alteration of hepatic function markers and lipid profile. Moreover, the degeneration of histoarchitecture of liver found in arsenic-treated rats was protected along with partial but definite prevention against DNA fragmentation induction. Similarly, generation of reactive oxygen species and free radicals were found to be significantly less along with restored activities of antioxidant enzymes in *M. oleifera* co-administered group with comparison to arsenic alone treatment group, which offers strong evidence for the hepato-protective and antioxidative efficiencies of *M. oleifera* extract against oxidative stress induced by arsenic. The hydro-alcoholic extract of *Moringa oleifera* was found to increase liver enzymes involved in the reactions of Phase I (cytochrome $b_5$ and cytochrome $p_{450}$) and Phase II (glutathione S-transferase) enzymes responsible for the detoxification of xenobiotic substances [106].

Results of Rakesh and Singh, [107] show that extract was effective in the reducing $CCl_4$ induced enhanced activities of SOD, GLU, Catalase, lipid peroxidation and % viability. Data shows that $CCl_4$ treatment decreased SOD, catalase, glutathioe, and peroxidase, while increased lipids oxidation and MDA, which is the by-product of lipid peroxidation. Pretreatment with hydro-alcohalic leaf extract of moringa olifera improved the SOD, catalase, glutathione, and peroxidase levels significantly($p<0.05$) and reduced lipids peroxidation. This shows hepatocellular damage caused by $CCl_4$ and its recovery by pretreatment with the crude extract of leaves and pods suggest that it might be considered as a potential source of natural antioxidant agent, which could be related to the free radical scavenging properties of various components present in varying concentration in the extract which is evident from the free radical measurement.

Elevated levels of total cholesterol, triglycerides, phospholipids and free fatty acids were restored to normal level by the administration of the leaf extract. This provides a scientific rationale to the traditional use of this plant in the management of liver diseases [108].

DNA ploidy study in this work proved that $CCl_4$ caused marked hypoploidy in hepatocytes, while treatment with moringa extract caused the DNA values became comparable with normal values. These results go in agreement with those of Sreelatha et al. [109], who stated that induction of apoptosis is the key success of plant products as anticancer agent and that moringa leaves extract showed a dose-dependent inhibition of cell proliferation associated with induction of apoptosis as well as morphological changes and DNA fragmentation. Thus, it is indicated that *Moringa oleifera* leaf extracts has potential for cancer chemoprevention and can be claimed as a therapeutic target for cancer.

They also coincided with those of Khalafalla *et al*. [14], as he reported that different extracts from the leaves of *Moringa olifera* could kill majority (70-86%) of the abnormal cells among primary cells harvested from 10 patients with acute lymphoblastic leukemia (ALL) and 15 with acute myeloid leukemia (AML) as well as a culture of hepatocarcinoma cells (75% death), but most significantly by the hot water and ethanol extracts..

## Conclusion:

In conclusion, the present study proved that MOLE is able to significantly alleviate the oxidative stress induced by $CCL_4$ in rats., These results revealed that Moringa oleifera has therapeutic effect in curing some health problems associated with toxication status (as a result of $CCL_4$ treatment) and this was established by its positive effect on some of molecular genetic, hematological, histopathological and histochemical parameters of the experimental animals.

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## References

1. Choi JH, Kim DW, Yun N, Choi IS, Islam MN, Kim YS, Lee SM. Protective effects. of hyperoside against carbon tetrachloride-induced liver damage in mice." Nat. Prod 2011; 74: 1055-1060.
2. Fakurazi S, Hairuszah I, Nanthini U. *Moringa oleifera Lam* prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chem. Toxicol 2008; 46: 2611- 2615.
3. Huang, B.; Ban, X.; He. J.: Tong, J.: Tian,I. and Wang. Y. (2010): Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (*Nelumho nucilero Gaertn*) leaves. Food Chem., 120: 873-878.
4. Fakurazi S, Sharifudin SA, Arulselvan P. *Moringa oleifera* hydroethanolic extracts effecively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules 2012; 17: 8334-8350.
5. Sabir SM, Rocha IB. Water-extractable phytochemicals from *Phyllanthus niruri* exhibit distinct *in vitro* antioxidant and *in vivo* hepatoprotective activity against paracetamol-induced liver damage in mice. Food Chem 2008; III: 845-851.
6. Bhaskar VH, Balakrishnan N. Protective effects of *Pergularia daemia* roots against paracetamol and carbon tetrachloride-induced hepatotoxicity in rats. Pharm. Biol 2010; 48: 1265-1272.
7. Nayak SS, Jain R, Sahoo AK. Plepatoprotective activity of *Glycosmis pentaphyllu* against paracetamol-induced hepatotoxicity in Swiss albino mice. Pharm Biol 2011; 49: 111-117.
8. Hausteen BH. The biochemistry and medical significance of the flavenoids. Pharmacology and therapeutics Journal 2005; 96: 67-202.
9. Nandakumar V, Singh T, Katuiyar S. Mutt- targeted prevention arid therapy of cancer by proanthocyanidins. Cancer Lett 2008; 269: 378-387.
10. Li-Weber, M. (2009): New therapeutic aspects of havones: the anticancer properties of Sculellaria and its main active constituents. Cancer Treat. Rev., 35: 57-68.
11. Dhawan V, Jain S. Garlic supplementation prevents oxidative DNA damage in essential hypertension. Mol. Cell Biochern 2005; 275: 85-94.
12. Ayinde B, Omogbai E, Arneaechina F. Pharmacolgnosy and hypotensive evaluation of Ficus exasperate Vaht (Moraceacl. leaf. Acta Pol. Pharrn 2007; 64(6): 543-6.
13. Kasolo, J. N.; Bimenya, C. S.; Ojok, L.; and Ogwal-okeng, J. W. (2011): Phytochemicals and acute toxicity of *Moringa oleifera* roots in mice. Journal of pharmacognosy and Phytotherapy, 3(3): 38-42.
14. Khalafalla, M. M.; Abdellatef, E.; Dafalla, H. M.; Nassrallah, A. A.; Aboul-Enein, K. M.; Lightfoot, D. A.; El-Deeb, F. E.; El-Shemy, H. A. (2010): Active principle from *Moringa oleifera Lam* leaves effective against two leukemias and a hepatocarcinoma. African Journal of Biotechnology, 9(49): 8467-8471.
15. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother Res 2007; 21: 17-25.

16. Bharali R, Tabassum J, Azad MRH. Chemomodulatory effect of Moringa oleifera, Lam, on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pacific Journal of Cancer Prevention 2003; 4: 131-139.
17. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem. Toxicol 2009; 47: 2196-2201.
18. Sreelatha S, Padma PR. Modulatory effects of *Moringa oleifera* extracts against hydrogen peroxide-induced cytotoxicity and oxidative damage. Hum. Exp. Toxicol 2011; 30: 1359-1368.
19. Makkar HPS, Becker K. Nutritional value and anti-nutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Animal Feed Sci Tech 1996; 63: 211-228.
20. Freiberger CE, Vanderjagt DJ, Pastuszyn A, Glew RS, Mounkaila G, Millson M, Glew RH. Nutrient contents of the edible leaves of seven wild plants from Niger. Plant Foods for Human Nutrition 1998; 53: 57-69.
21. Nambiar VS, Seshadri S. Bioavailability trials of i-carotene from fresh and dehydrated drumstick leaves (*Moringa oleifera*) in a rat model. Plant Foods for Human Nutrition 2001; 56: 83-95.
22. Lakshminarayana, R.; Raju, M.; Krishnakantha. T. P. and Baskaran, V. (2005): Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. J Agri Food Chem.; 53:2838-2842.
23. Sanchez MDI, Lopez CJ, Vazquez NJR. A- high-performance liquid chromatography method to measure 2- and i-tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. J. Chrom2006; 1105: 111-114.
24. Chuang PH, Lee CW. Chou JY, Murugan M, Shieh BJ, Chen HM. Antifungal activity of crude extracts and essential oils of *Moringa oleifera Lam*. Biores Tech 2007; 98: 232-236.
25. Nepolean P, Anitha J, Emilin RR. Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera Lam*. Current Biotica 2009; 3 (1):33-37.
26. Rao AV, Devi PU, Kamath R. *In vivo* radioprotective effect of *Moringa oleifera* V leaves. Indian J Exp Biol 2001; 39(9): 858-63.
27. Eshak MG, Osman HF. Role of *Moringa oleifera* leaves on biochemical and genetical alterations in irradiated male rats. Middle-East Journal of Scientific Research 2013; 16(10): 1303-1315.
28. Sathya TN, Aadarsh P, Deepa V, Balakrishna MP. *Moringa oliefera Lam*, leaves prevent Cyclophosphamide-induced, micronucleus and DNA damage in mice. International Journal of phytomedicine 2010; 2: 147-154.
29. Pari L, Kumar NA. Hepatoprotcetive activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J Med Food 2002; 5: 171-177.
30. Monica PHK, Sharma B, Sarkar C, Singh C. Kinetics of drumstick leaves(*Moringa oleifera*) during convective drying. African Journal of Plant Science 2010; 4 (10): 391-400.
31. Patel RK, Manish MP, Nilesh RK, Kirit RV, Patel RK. *In vitro* hepatoprotective activity of *Moringa oleifera Lam*. Leave on isolated Rat hepatocytes. Int J Ph Sci 2010; 2(1): 457-463.
32. Kumar, P. V.; Sivaraj, A.; Elumalai, E. K. and Kumar, B. S. (2009): Carbon Tetrachloride-Induced Hepatotoxicity in Rats. Protective Role of Aqueous Leaf Extracts of *Coccinia Grandis*. International Journal of Pharm. Tech. Research, 1(4): 1612-1615.
33. Castro JA, De Ferreyra EC, De castro CR, Fenoes OM, Sasame H, Gillette JR. Prevention of carbon tetrachloride-induced necrosis by inhibitors of drug metabolism-further studies on their mechanism of action. Biochem pharmacol 1974; 23:295-302.
34. Pang S, Xin X, Pierre MV. Determine of metablic disposition. Ann. Rev. Pharmacol. Toxicol 1992; 32: 625-626.
35. Abdou HS, Salah SH, Booles HF. Abdel Rahim EA. Effect of pomegranate pretreatment on genotoxicity and hepatotoxicity induced by carbon tetrachloride ($CCL_4$) in male rats. Journal of Medicinal Plants Research 2012a; 6(17): 3370-3380.
36. Abdou HS, Salah SH, Booles HF, Abdel Rahim EA. Antioxidant effect of celery against carbon tetrachloride induced hepatic damage in rats, African Journal of Microbiology Research 2012b; 6(27): 5657-5667.
37. Palanivel MG, Raykapoor B, Kumar RS, Elinstein JK, Kumar EP, Kumar MR, Kavithan K, Kumar MP, Jayakar B. Hepatoprotective and antioxidant effect of *Pisonia aculeate L*. against $CCL_4$-induced hepatic damage in rats. Sci Pham 2008; 76: 203-215.
38. Ugwu OP C, Nwodo O FC, Joshua PE, Abubakar B, Ossai EC, Christian O. Phytochemical and acute toxicity studies of *Moinga oleifera* ethanol leaf extract. Int. J. Life. Sc. Bt & Pharm. Res 2013; 2(2): 65-71.
39. Paget GE, Barnes JM. Toxicity tests. In: Laurence DR, Bacharach Al (ed.) Evaluation on drug activities. Pharmacometrics, London, Academic Press1964; p:161.

40. Abdel Salam OME, Sleem AA, Shafee N. Hepatoprotective effects of the nitric oxide donor isosoribde-5-mononitrate alone and in combination with the natural hepatoprotectant, silymarin, on carbon tetrachloride-induced hepatic injury in rats. Inflammopharmacol 2010; 18: 87-94.
41. Abdel Salam OME. Sleem AA, Shafee N. Effect of Crataegus extract on carbon tetrachloride-induced hepatic damage. Comp Clin. Pathol. Doi 2011; 10.1007/s00580-011-1356-7.
42. Blasiak J, Arabski M, Krupa R, Worzniak K, Zadrozny M, Kasznicki J, Zurawaska M, Drzewoski JJ. DNA damage and repair in type 2 diabetes mellitus. Mutation Research 2004; 554: 297-304.
43. Kronmiller, J. E.; Nguyen, T.; Berndt, W. and Wickson, A. (1995). Spatial and temporal distribution of Sonic hedgehog mRNA in the embryonic mouse mandible by reverse transcription/polymerase chain reaction and *in situ* hybridization analysis. Arch Oral Biol., 40: 831-838.
44. Dacice JV, Lewis SN. Practical Haematology (6tyh ed). Chruchill Livingstone, Edinburg 1984; pp: 112.
45. Merghani TH. The Core of Medical Physiology 3`d ed., 2010; pp:157 – 158.
46. Drury RAB, Walington EA. Carlton's Histological Technique, 5th Ed, Oxford, Oxford University press, Oxford UK 1980.
47. Feulgen R, Rosenbek HC. Manual of histological demonstration technique. Butter Worth & Co (publishers) Ltd. London, Herford, Haverhill 1942.
48. Danque PD, Chen HB, Patil J, Jagidar J, Orsatti G, Paronetto F. Image analysis versus flow cytometry for DNA ploidy quantitation for tumors: A comparison of six methods of sample preparation. Mod Pathol 1993; 6: 270-275.
49. SAS Institute, Inc. User's Guide: Statistics. SAS Institute. Cary NC, 1982.
50. Waller RA, Duncan DB. A Bayes rule for the symmetric multiple comparison problem. J. Am State. Assoc 1969; 64: 1484-1503.
51. Eastman A, Barry MA. The origins of DNA breaks: a consequence of DNA damage, DNA repair, or apoptosis? Cancer Invest 1992; 10(3):229-240.
52. Gruebele A, Zawaski K, Kaplan D, Novak R. Cytochrome $P_{450}$ 2E1- and cytochrome $P_{450}$2B1/2B2-catalyzed carbon tetrachloride metabolism: effects on signal transduction as demonstrated by altered immediate-early (c-Fos and c-Jun) gene expression and nuclear AP-1 and NFKB transcription factor levels. Drug Metabol Disp 1996; 24: 15-22.
53. Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsumi VK. Kupffer cell inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp. Biochem. Physiol. C Toxicol Pharmaco 2001; 130: 219-226.
54. Weber LWD, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Critical Reviews in Toxicology 2003; 33 (2): 105136.
55. Saba AB, Oyagbemi AA, Azeez OI. Ameioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of *Cnidoscolus conitifolius* in rats. Nig. J. Physiol Sci 2010; 25: 139-147.
56. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutat Res 2002; 521:151-163.
57. Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH. Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. Environ Toxicol Chem 1999; 18: 2275-2282.
58. Soini Y, Chia SC, Bennett WP. An aflatoxin-associated mutational hotspot at condon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinomas from Mexico. Carcinogenesis1996; 17: 1007-1012.
59. Harris CC. The 1995 Walter Hubert lecture-molecular epidemiology of human cancer: insights from the mutational analysis of the p53 tumor-suppressor gene. British Journal of Cancer 1996; 73: 261-269.
60. Mace, K.; Aguilar, F.; Wang, J. S.; Vantavers, P.; Gömez-LechOn, M.; Gonzalez, F. J.; Croopman, J.; Harris, C. C.; and Pfeifer, M. A. (1997): Aflatoxin BI-induced DNA adduct formation and P53 mutations in CYP450-expressing human liver cell lines. Carcinogenesis, 18: 1291-1297.
61. Huang, M. E. and Kolodner, R. D. (2005): A biological network in *Saccharomyces cerevisiae* prevents the deleterious effects of endogenous oxidative DNA damage. Molecular Cell, 17: 709-720.
62. Guardiola F, Ricard B, Rafel C, Alba T, Tric A. Determination of hydroperoxides in food sand biological samples by the ferrous oxidation-xylenol orange method: a review of the factors that influence the method's performance. Analytical Biochemistry 2008; 377: 1-15.
63. Roberts RA, James NH, Cosulich S, Hasmall SC, Orphanides G. Role of cytokines in nongenotoxichepatocarcinogenesis: cause or effect? Toxicol Lett 2001; 120: 301-306.

64. Tahashi Y, Matsuzaki K, Date M, Yoshida K, Furukawa F, Sugano Y, Matsushita M, Himeno Y, Inagaki Y, Inoue K. Differential regulation of TGF-B signal in hepatic stellate cells between acute and chronic liver injury. Hepato 2002; 135: 49-61.
65. Waxman DJ, Azaroff L. Phenobarbital induction of cytochrome $P_{450}$ gene expression. Biochem. J 1992; 281 (3): 577-592.
66. Wyde ME, Bartolucci E, Ueda A, Zhang H, Yan B, Negishi M, You L. The environmental pollutant 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene induces rat hepatic cytochrome $P_{450}$ 2B and 3A expression through the constitutive androstane receptor and pregnane X receptor. Mol. Pharmacol 2003; 64: 474-481.
67. Pan J, Xiang Q, Ball S. Use of a novel real-time quantitative reverse transcription-polymrase chain reaction method to study the effects of cytokines on cytochrome on cytochrome P450 mRNA expression in mouse liver. Drug Metab. Dispos 2000; 28: 709-713.
68. Sun G, Thai SF, Tully DB, Lambert GR, Goetz AK, Wolf DC, Dix DJ, Nesnow S. Propiconazole-induced cytochrome $P_{450}$ gene expression and enzymatic activities in rat and mouse liver. Toxicology Letters 2005; 155: 277-287.
69. Eshak MG, Hassanane MM, Farag IM, Fadel M. Bioremedination of polluted diets with industrial wastewater using marine and earth fungi: molecular genetic, biochemical and histopathological studies in mice. World Applied Sciences Journal 2012; 20(2): 290-304.
70. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variation human liver cytochrome $p_{450}$ enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther 1994; 270, 414-423.
71. Kimura, S.; Kawabe, M.; Ward, J. M.; Morishima, H.; Kadlubar, F. F.; Hammons, G. J.; Fernandez-Salguero, P. and Gonzalez, F. J. (1999): CYP1A2 is not the primary enzyme responsible for 4-aminobiphenyl-induced hepatocarcinogenesis in mice. Carcinogenesis, 20: 1825-1830.
72. Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. Mol Pharmacol 2002; 62: 638-646.
73. Kobayashi, K.; Urashima, K.; Shimada, N. and Chiba, K. (2002): Substrate specificity for rat cytochrome P450 (CYP) isoforms: screening with cDNA-expressed systems of the rat. Biochem. Pharmacol., 63: 889-896.
74. Honkakoski P, Sueyoshi T, Negishi M. Drug-activated nuclear receptors CAR and PXR. Ann. Med 2003; 35: 172-182.
75. Wilson TM, Kliewer SA. PXR, CAR and drug metabolism. Nat. Rev. Drug Discov 2002; 1: 258-266.
76. Prasanna V, Sreelatha S. Synergistic effect of *Moringa oleifera* attenuates oxidative stress induced apoptosis in *Saccharomyces cerevisiae* cells: evidence for anticancer potential, Int J. Pharm Bio Sci, 2014; 5(2): 167-177.
77. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology, Cancer Causes Control1991a; 2:325-357.
78. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. II. Mechanisms, Cancer Causes Control 1991b; 2: 427-442.
79. Iqbal, S. and Bhanger, M. I. (2006): Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J Food Comp Anal., 19: 544-551.
80. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera Lam* Leaves. J Ethnopharmacol 2008; 116: 439-446.
81. Duthie SJ, Ma A, Ross MA, Collins AR. Antioxidant supplementation decrease oxidative DNA damage in human lymphocytes. Cancer Res 1996; 56:1291-1295.
82. Valko M, Rhodes CJ, Moncol J, Izakovic M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Mini-review.Chem. Biol. Interact 2006; 160: 1-40.
83. Ezejindu DN, Chinweife KC, Thentuge CJ. The effects of moringa extract on liver enzymes of carbon tetrachloride induced hepatototxicity in adult wister rats. The International Journal of Engineering and Sciences (IJES) 2013; 2(7): 54-59.
84. Bennett RN, Mellon FA, Foidl N, Pratt JH, DuPont MS, Perkins L, Kroon PA. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera L.* (Horseradish tree) and *Moringa stenopetala L.* J. Agri. Food Chem 2003; 3546-3553.
85. Fahey JW, Dinkova-Kostova AT, Talalay P. The "Prochaska" microtiter plate bioassay V for inducers of NQ01. Chapter 14 in Methods in Enzymology, (Eds.) H. Sies & L. Packer, Elsevier Science, San Diego, CA., 2004; 382(B): 243-258.

86. Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito Y, Tokuda H, Nishino H. An antitumor promoter from *Moringa oleifera Lam*. Mutat Res 1999; 440: 181-188.
87. Sule OJ, Elekwa I, Ayalogu EO. Effect of *Acalypha wilkesiana muell arg*. on haematological parameters in wistar albino rats. Int J Biol. Med Res 2012; 3(1): 1234-1237.
88. Elshater AA, Salman MMA, Moahmed S. The hepato-ameliorating effect of *Solanum nigrum* against $CCL_4$ induced liver toxicity in Albino rats. Egypt Acad J Biolog Sci 2013; 5(10): 59-66.
89. Britton RS, Bacon BR. Role of free radicals in liver diseases and hepatic fibrosis. Hepatogastroenterol 1994; 41: 343 - 348.
90. Moritz RP, Pankow D. Effect of carbon tetrachloride and chloroform on hematologic parameters in rats. Folia Haematollnt Mag Klin Morphol Blutforsch 1989; 116 (2): 283-287.
91. Jain, N. C. (1986): Schalm's Veterinary Haematology 4th ed. Lea and Febiger, Philadelphia, USA, pp: 564-572.
92. Essawy AE, Hamed SS, Abdel-Moneim AM, Abou-Gabal AA, Alzergy AA. Role of black seeds (*Nigella sativa*) in ameliorating carbon tetrachloride induced haematotoxicity in Swiss Albino mice. Journal of Medicinal Plants Research 2010; 4(19): 1977-1986.
93. Maduka HCC, Okoye ZSC, Ladeji O, Egbe PE. The protective role of *Sacoglottis gabonensis* stem bark extract against peroxidation reactions *in vivo* and *in vitro*. Nigeria Journal of Biotechnology 1999; 10: 1-8.
94. Maduka HCC, Okoye ZSC. The effect of *Sacogglotettis gabeonensis* beverage additive on the natural antioxidant defenced during 2, 4-dinitrophenyl hydrazine-induced membrane peroxidation *in vivo*. Vasc Pharm 2002; 39: 21-31.
95. Maduka HCC, Okoye ZSC, Eje A. The influence of *Sacoglottis gabonensis* stem bark extract and bergenin its isolate, Nigerian alcoholic beverage additives on the metabolic and haematological side effect 2,4-dinitrophenyl hydrazine-induced tissue damage. Vasc. Pharm 2003; 39(6): 317-324.
96. Maduka HCC; Daja A, Gadaka GA, Ugwu CE, Dike CC, Abubakar KA, Maduka AA. Protective role of *Moringa oleifera lam* aqueous leaf extract on some excretory products and hematological parameters in acetaminophen induced albino rats. IOSR Journal of Nursing and Health Science (IOSR-JNHS) 2014; 3(2): 27-31.
97. Patrick-lwuanyanw, KC, Wegwu MO, Ayalogu EO. Prevention of CC14 induced liver damage by ginger, garlic and vitamin E. Pakistan J. Bio. Sci 2007; 10(4): 617-621.
98. Oluyemi KA, Omotuyi IO, Jimoh OA, Saalu CL, Josiah SJ. Erythropoetic and antiobesity effects of *Garcinia cambogia* (bitter kola) in Wister rats. Biotechnol Appl Biochem 2007; 46: 69-72.
99. Mada, S. B.; Inuwa, H. M.; Abarshi, M. M.; Mohammed, H. A. and Aliya, A. (2014): Hepatoprotective effect of *Momordica charantia* extract against $CCL_4$ induced liver damage in rats. British Journal of Pharmaceutic Research, 4(3): 368-380.
100. Chinwe C, Isitua N. Studies on the haematological impact of *Moringa oleifera* in rabbits. A poster presented at 2'd Intemationals Conference on Applied Biotechnology, 2010; October 2527, Khartoum, Sudan.
101. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant foods Hum Nutr 2009, 64: 303-311.
102. Osman HM, Shayoub ME, Babiker EM. The Effect of *Moringa oleifera* Leaves on Blood Parameters and Body Weights of Albino Rats and Rabbits. Jordan Journal of Biological Sciences 2012; 5(3): 147-150.
103. Bertling I, Odindo AO, Workneh TS, Mathaba N. Levels of anti-oxidants in different parts of moringa seedling. African Journal of Agricultural Research 2011; 6 (22): 5123-5132.
104. Hamza AA. Ameliorative effects of Moringa oleifera Lam seed extract on liver fibrosis in rats.Food and Chemical Toxicology 2010; 48(1): 345-355.
105. Chattopadhyay S, Maiti S, Maji G, Deb B, Pan B, Ghosh D. Role of Protective Moringa oleifera (Sajina) seed on arsenic-induced hepatocellular degeneration in female albino rats. Biological Trace Element Research 2011; 142(2): 200-212.
106. Chadamas P, Piegnchai K, Siriporn T, Chaniphun B. Nutritive Evaluation and Effect of Moringa oleifera pod on clastogenic Potential in the Mouse. Asian Pacific J of Cancer Prev 2010; 11: 627-63.
107. Rakesh S, Singh VJ. *In vivo* antioxidant activity of *Moringa oleifera* leaf and pod extracts against carbon tetra chloride induced liver damage in albino mice. Journal of Chemical and Pharmaceutical Research 2010; 2(6): 275-283.
108. Sreelatha S, Padma PR. Hepatoprotective potential of *Moringa oleifera* leaves against ethanol-$CCL_4$ induced hepatic injury in experimental rats. Plant Archives 2008; 8(2): 647-655.

109. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. Food and Chemical Toxicology 2011; 49 (6): 1270-1275.

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