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Moringa oleifera leaf Extract: A Potent Ameliorator of Cyclophosphamide Induced liver Toxicity in Rat Model

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Abstract

Moringa oleifera leaf is a pharmacologically active with documented antioxidant activity. In the current study protective effect of ethanol extract of *Moringa oleifera* leaf extract (MLE) was investigated in rats against cyclophosphamide (CYP) induced liver injuries. Twenty eight Wistar albino rats were divided into four groups, as follows: 1) control group - received vehicle used for MLE and CYP for 14 days; 2) MLE group - rats were administered orally at a dose 200.0 mg kg $^{-1}$ b.wt. for 14 consecutive days; 3) CYP group - cyclophosphamide at a dose of 150 mg/kg was given through i.p. to rats as a single dose at day 7; 4) MLE + CYP group – MLE was given for 14 days plus a single dose of CYP was given on hour after MLE administration. Catalase (CAT), glutathione (GSH), the level of lipid peroxidation thiobarbituric acid-reactive substances (TBARS), DNA and RNA concentration were analyzed in liver tissue. In addition, serum total protein, albumin, cholesterol and triglycerides, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) values were evaluated. AST, ALT, ALP, LDH, triglycerides and total cholesterol in serum were found to be significantly ($P < 0.05$) higher in CYP group, compared to control group, while protein and albumin were decreased. Compared with the control group, significantly high levels of liver TBARS and the low antioxidant defenses, like free radical scavenging enzyme viz., catalase activity as well as GSH concentration in CYP-treated group. In rats supplemented with MLE as well as treated with CYP, hepatic specific marker enzymes were restored to normalcy which otherwise was lowered in the CYP-treated rats. In conclusion, MLE exhibited antioxidant activity by the presence of free radical quenching constituents.

Key Words: cyclophosphamide; *Moringa oleifera* leaf extract; Liver; Rat; Oxidative stress

1. Introduction

Free radicals are the major by-products produced by the cells of aerobic organisms and can start the autocatalytic reactions and spread the chain of damage by reacting with molecules and converting them into free radicals. Free radicals are mainly produced from these sources in the body: ubisemiquinone in the mitochondrial membrane, xanthine oxidase of endothelial cells, and myeloperoxidase and NADPH oxidase of neutrophils. But, xanthine oxidase and respiratory chain of mitochondria are the major sources of reactive oxygen species (ROS) (Akhlaghi and Bandy, 2009). Free radicals are extremely unstable and become stable by pairing an outer shell electron with biomolecules, for example, lipids, proteins, and DNA. At high concentrations, free radicals can cause damage to various cell structures, comprising proteins and nucleic acids, together with lipid peroxidation (Poli *et al.*, 2004; Abraham *et al*., 1999). These injuries and damages are the major contributions towards aging, cardiovascular diseases, atherosclerosis, cancer, and inflammatory diseases (Hazra *et al*., 2008). Living cells contain a defensive system of antioxidants against the ROS which avoids their unnecessary production and inactivate them. Various antioxidants have been reported to protect the body from the damaging effects of oxidative stress. Nowadays, research has been increased in the area of finding novel natural antioxidants due to low side effects in comparison to synthetic antioxidants (Gorinstein *et al*., 2003).

Cyclophosphamide (CYP), Figure 1a, is a synthetic alkylating agent chemically related to the nitrogen mustards (Takimoto *et al.*, 2005) widely used as an anticancer and immunosuppressive drug (Clern and Bickers, 1991; Paul and Bruce, 1991) and in the treatment of nephrotic syndrome (Kirkland *et al.*, 1976; Etteldorf *et al.*, 1976). It is effective against a wide spectrum of malignancies, such as, leukemia, lymphoma, breast, lung, prostate, and ovarian cancers (Khan *et al*., 2004; Shanafelt *et al.*, 2007). Many anticancer drugs are known for the generation of Reactive Oxygen Species (ROS) in cancer cells (Hanane *et al*., 2012) and these ROS generated lead to oxidative damage in the cell (Maiti, 2012). During bio activation of CYP, reactive oxygen species are also formed, which can modify the components of both healthy and neoplastic cell leading to decreased antioxidative capacity (Stankiewicz *et al*., 2002). CYP's antineoplastic effects are associated with phosparamide mustard, while acrolein is linked with its toxic side effects (Kern and Kehrer, 2002). The cytotoxic effects of CYP and other chemotherapeutic drugs result in part from their interaction with DNA leading to defective DNA, abnormal cell function and cell death (Lee and Schmitt, 2009). Several studies suggest that antioxidant supplementation can influence the response to chemotherapy as well as the development of adverse side effects that result from treatment with antineoplastic agents (Weiji *et al*., 1997).

The best source to get a range of novel herbal drugs is medicinal plants. *Moringa oleifera* is called "Miracle Vegetable" because it is both a medicinal and a functional food (Verma *et al*., 1976).

Moringa oleifera has the highest proportion of essential amino acids and significant quantities of minerals (Sabate, 2003) when analyzed. *Moringa oleifera* is rich in compounds like glucosinolates and isothiocyanates (Fahey *et al.*, 2001). Flowers contain pigments such as alkaloids, kaempferol, rhamnetin, isoquercitrin and kaempferritin (Faizi *et al.*, 1994). Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. Consequently, the aim of the present study was to investigate the antioxidant and chemo protective effect of MLE against CYP-induced oxidative stress in rats.

2. Materials and Methods

2.1. Chemicals

Cyclophosphamide, reduced glutathione (GSH), bovine serum albumin (BSA), All reagents were of the highest quality. Reduced glutathione, 1-chloro-2, 4-dinitrobenzenPyt, thiobarbituric acid (TBA), potassium iodide, sodium hydroxide, and trichloroacetic acid (TCA) and all other chemicals were purchased from Sigma Chemical Company (Saint Louis, USA).

2.2. Preparation of Extract

Moringa oleifera leaf powder (were supplied by Ancient Greenfields Ltd, Coimbatore-641108, INDIA). The aqueous extract was prepared as described by Thilza *et al.* (2010). 100 g of ground plant material (Moringa leaf) was macerated in 1.5 L of boiled distilled water for one hour. The mixture was filtered through Whatman filter paper No. 3 and filtrate obtained was evaporated to dryness using a rotator evaporator at 45°C. The extract obtained (22, 9% yield) was stored at 4°C. Extract solution was prepared in distilled water each time prior to experimentation.

2.3. Experimental Design

Male Sprague Dawley rats (160–170 g) of seven weeks old were used as animal model in this study. They were maintained in cages at room temperature of 23 ± 2 ºC with a 12 h light/dark cycle and free access to water and feed. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments. The European Community Directive (86/609/EEC) and National rules on animal care have been followed. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee. Permission to handling with laboratory animals was obtained from the Institute's animal committee. During the experiments, maximum care was taken to minimize animal suffering and, in addition, the number of rats used was kept at minimum. Twenty male rats were randomly distributed into 4 groups (5 rats/group):

- Group I (control): Rats received vehicle used for MLE and CYP for 14 days.
- Group II (Moringa leav extract (MLE)): Rats were administrated orally with MLE 200 mg/kg/day (Aja *et al.*, 2015) was administered orally through an intragastric tube every day and continued until rats were sacrificed) for 14 days.
- Group III (cyclophosphamide (CYP): CYP at a dose of 150 mg/kg (Kim *et al.*, 2013) was given through i.p. to rats.
- Group IV (MLE + CYP): received MLE for 7 consecutive days, before and after CYP injection at a dose of 150 mg/kg was injected once on day 7, 1 h after MLE administration

2.4. Animal Dissection

After last treatment, rats were unfed for 24 h. Diethyl ether was used to anesthetize animals and then dissected the animals from ventral side of the body. Blood was collected by piercing heart. Blood was collected in two types of tubes; that is, for serum analysis it was stored in small falcon tubes which was then centrifuged to obtain serum; the rest of the blood was collected in EDTA containing tubes for whole blood analysis. From the dissected animal, livers were removed and placed in saline solution. Liver was stored in liquid Nitrogen at −70°C for antioxidant enzymes and tissue stress marker examination.

2.5. Serum Analysis

After dissection of animals, blood was collected in falcon tubes and centrifuged at 4000 rpm for 20 min at 4°C, after 30 minutes, to collect the serum samples. Serum biomarkers were determined using a commercial kit in accordance with manufacturers' instructions using a spectrophotometer (Shimadzu UV-VIS Recording 2401 PC, Japan). Serum samples were analyzed for total protein by the Biuret method according to Armstrong and Carr (1964). Albumin concentration was determined by the method of Doumas *et al.* (1977). Globulin concentration was determined as the difference between total protein and albumin. Serum alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were determined using commercial kits obtained from Biodiagnostic kit (Cairo, Egypt). The principle reaction of the colorimetric determination of AST or ALT activity is based on the reaction of aspartate or alanine with αketoglutarate to form oxaloacetate or pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine (Reitman and Frankel, 1957). Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenylphosphate as a substrate (Rosalki and Foo, 1993). Serum lactate dehydrogenase (LDH; EC 1.1.1.27) was determined according to the method of Friedman and Young (1997), using kit obtained from Spinreact (Santa Coloma, Spain), Also, low density lipoprotein (LDL) cholesterol was measured according to the method of Assmann *et al.* (1984), high density lipoprotein (HDL) cholesterol was measured according to the method of Burstein *et al.* (1984). Cholesterol and triglycerides was measured according to the method Carr *et al.* (1993) using Biodiagnostic kit (Cairo, Egypt).

2.6. Lipid peroxidation, antioxidant non-enzymatic and enzymatic estimation in liver tissue

Tissue homogenate was prepared by homogenizing 100 mg of liver tissue in 1 mL of 100 mM potassium phosphate buffer containing 1 mM EDTA at pH 7.4. Supernatant was collected in clean falcon tubes after centrifugation for 30 min at 12000 ×g at 4°C and was used for further analysis. The following assays were carried out to analyze the pharmacological activity against the toxicity induced with CYP in rats.

Lipid peroxidation assay

Using TBA, thiobarbituric acid reactive substances (TBARS) were measured in the tissue homogenate (Ohkawa *et al.*, 1979). An aliquot of 100 µL of tissue homogenate was added to 100 µL of 100 mM ascorbic acid, 10 µL of 100 mM $FeCl_3$, and 290 µL of sodium phosphate buffer (pH 7.4). Reaction solution was incubated for 1 hour in a shaking water bath at 37°C. Addition of 500 µL of 10% TCA stopped the reaction. Place reaction tubes were placed in boiling water bath for 15 minutes after adding 500 µL of 0.67% TBA. After 15 min tubes

were shifted on a crushed ice for 5 minutes and centrifuged for 10 minutes at 2500 ×g. Optical density of supernatant was measured at 535 nm to determine the amount of TBARS formed. Using molar extinction coefficient of 1.560 × 10^5/M/cm, lipid per oxidation activity was measured as an amount of TBARS formed/min/mg tissue.

Reduced glutathione

Reduced glutathione (GSH) activity was measured according to method of Jollow *et al.* (1974). 500 µL of tissue homogenate was precipitated by addition of 500 µL of sulfosalicylic acid (4%). After 1 hour incubation at 4°C, samples were centrifuged for 20 min at 1200 ×g. 33 µL of supernatant was collected and added to aliquots containing 900 µL of 0.1 M potassium phosphate buffer (pH 7.4) and 66 µL of 100 mM DTNB. Reduced glutathione reacts with DTNB and forms a yellow colored complex. Absorption was measured at 412 nm. µM GSH/g tissue represents GSH activity

Catalase (CAT) activity

Measurement of CAT (EC. 1.11.1.6) activity is based on the methodology of Shah *et al.* (2013), which relies on decomposition of H_2O_2. An aliquot of 25 µL of tissue homogenate was added to 100 µL of 10 mM H_2O_2 and 625 µL of 5 mM EDTA buffer (pH 8.0). The disappearance of H_2O_2 in the reaction mixture by catalase was measured spectrophotometrically at 230 nm. CAT activity was expressed as U/g tissue.

2.7. Measuring RNA and DNA concentration

The RNA concentration in the samples was determined by precipitation of the nucleic acids in 0.5 M $HClO_4$, after which the RNA was hydrolyzed ice by incubation in 0.3 M KOH at 60°C for 1 h. After preremoval of DNA by 0.5 M $HClO_4$ precipitation, the RNA concentration was determined by boiling the samples for 30 min in 6 M HCl, 0.01% $FeCl_3$ and 0.3% orcinol. Absorption was measured at 660 nm. RNA concentrations were calculated using a calibration curve with highly purified total adult heart RNA that was isolated by ultracentrifugation through a caesium chloride cushion (Chirgwin *et al.*, 1979) as a standard.

The DNA concentration in the samples was determined by hydrolyzing the RNA in 0.1 M NaOH after which the DNA was precipitated with half volume 10% $HClO_4$. The DNA was suspended in 10% 32P$HClO_4$ and incubated at 70°C to hydrolyze the DNA. After clearing the solution by centrifugation, dip- henylamine and acetaldehyde were added to a final concentration of 2% and 0.01%, respectively. After an overnight incubation at 30°C the absorbance was determined at 560 and 700 nm. The concentration in the samples was calculated relative to the included calibration curve that was prepared using highly Mopurified herring testis DNA (Sambrook *et al.*, 1989).

2.8. Statistical Analysis

Data are expressed as mean ± S.E.M. (Significance was calculated at $P<0.05$). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test (Daniel, 1991).

3. Results

The present experiment was carried out to determine the protective role of MLE against CYP induced liver toxicity at biochemical level in rats.

3.1. Estimation of cyclophosphamide toxicity on serum protein, albumin, and globulin

Cyclophosphamide treatment in rats caused significant change in the serum protein

profile. To analyze the protective effect of MLE, the fluctuations in serum total protein, albumin, and globulin were analyzed (Table 1). A significant difference was observed in CYP treated group in comparison to control group. MLE treatment showed protective role by significantly (P <0.05) decreasing its level in the serum.

3.2. Hepatic toxicity

Liver function tests commonly include tests to measure levels of several enzymes, which are special proteins that help the body break down and metabolize other substances. Enzymes that are often measured include ALT; AST and ALP (Berk and Korenblat, 2007). The protective effect of MLE on CYP – induced changes in serum ALT, AST and ALP in rats was presented in Table (1). Treatment with CYP significantly (P<0.05) increased the activities of AST, ALT, ALP and LDH in serum compared to control animals. The present study demonstrates that treatment with MLE alone did not cause any significant change in enzyme activities in serum and alleviated the toxicity of CYP. MLE in combination with CYP alleviated its negative effect on activities of the above measured enzymes (Table 1).

Table 1 Effects of Moringa leaf extract on biochemical parameters of serum from Cyclophosphamide-treated rats

Parameters	Groups			
	Control	MLE	CYP	MLE + CYP
Total protein (g/dl)	9.64 ± 0.69	9.88 ± 0.53	7.2 ± 0.97 [abd]	8.62 ± 0.25 [abc]
Albumin (g/dl)	4.38 ± 0.09	4.47 ± 0.13	4.13 ± 0.1 [abd]	4.41 ± 0.06 [c]
Globulin (g/dl)	5.86 ± 0.37	5.88 ± 0.24	3.3 ± 0.26 [abd]	4.78 ± 0.34 [abc]
AST (U/L)	122.2 ± 8.2	113.6 ± 2.72	310.0 ± 16.44 [abd]	209.6 ± 17.17 [abc]
ALT (U/L)	31.8 ± 1.7	27.4 ± 2.05	120.6 ± 013.06 [abd]	61.8 ± 17.08 [abc]
ALP (U/L)	154.0 ± 12.0	129.2 ± 12.9[acd]	309.0 ± 24.5 [abd]	215.6 ± 19.9[abc]
LDH (U/L)	1096.8 ± 81.4	918.6 ± 47.3[acd]	1722.2 ± 152.9 [abd]	1378.2 ± 67.3[abc]

Values are mean ± SE of five rats in each group. Significance at P <0.05. MLE: Moringa leaf extract; CYP: Cyclophosphamide

[a] Compared of control and other groups

[b] Compared of MLE group and other groups

[c] Compared of CYP group and other groups

[d] Compared of MLE+CYP group and other groups

3.3. Lipid and lipoprotein profiles

Data represented in Table 2 show that i.p. treatment with CYP caused significant (P<0.05) increase in serum cholesterol, triglycerides and LDL concentrations, whilst HDL levels were decreased compared to control animals. MLE alone caused significant (P<0.05) decrease in lipid profile compared to control. MLE-pretreated to rats treated with CYP showed reduction in the serum lipid level caused by CYP (Table 2).

Table 2: Effects of Moringa leaf extract on lipid and lipoprotein profiles of serum from

Cyclophosphamide-treated rats

Parameters (mg/dl)	Groups			
	Control	MLE	CYP	MLE + CYP
Triglycerides	65.9 ± 3.08	52.48 ± 3.84[acd]	111.08 ± 13.16 [abd]	87.68 ± 3.8[abc]
Total cholesterol	50.98 ± 3.98	42.26 ± 1.8[acd]	87.16 ± 6.08 [abd]	63.68 ± 5.85[abc]
HDL	33.05 ± 2.1	37.08 ± 1.28[acd]	20.86 ± 2.81 [abd]	27.61 ± 1.06[abc]
LDL	14.42 ± 0.78	11.72 ± 1.0[acd]	35.76 ± 5.09 [abd]	20.8 ± 2.84[abc]

Values are mean ± SE of five rats in each group. Significance at P <0.05. MLE: Moringa leaf extract; CYP: Cyclophosphamide; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol.

[a] Compared of control and other groups
[b] Compared of MLE group and other groups
[c] Compared of CYP group and other groups
[d] Compared of MLE+CYP group and other groups

3.4. Lipid peroxidation, antioxidant non-enzymatic and enzymatic estimation in liver tissue

In order to characterize the protective effect of MLE, change in oxidant and antioxidant enzyme level was evaluated after CYP treatment. Figures 1-3 shows the protective effect of MLE on liver tissue TBARS, GSH and CAT. MLE per se had no effect on serum levels of TBARs when it compared with controls. In CYP treated rats, a significant (P<0.001) increase of lipid peroxidation in serum was observed, as evidenced by the enhanced TBARs levels in serum compared with those of controls. With MLE pretreatment in CYP-treated rats, a dramatic reduction (P<0.05) in TBARs levels was recorded compared to CYP-treated group (Figure 1).

In comparison to control group, the levels of GSH and CAT in liver tissues were considerably decreased (P<0.05) after the CYP treatment. MLE co-treatment reversed the activity of these non-enzymatic and enzymatic antioxidant towards normal (Figures 2, 3).

3.5. Liver DNA and RNA concentration

Cyclophosphamide decreased (P<0.05) liver DNA and RNA concentrations compared to the control. The combination of MLE and CYP was less effective to DNA and RNA compared to individual treatment of CYP (Figure 4).

4. Discussion

Regardless of the enormous advancement in the field of pharmacology and conventional chemistry in creating effective medicines, still plant kingdom is a reservoir of natural therapeutics and offers a valuable source of novel drugs and medicinal entities. Medicinal plants and their phytochemicals are the main source of herbal drugs that can affect the physiological system of animals either directly or indirectly. Plant-based medicines have minimal or no side effects; therefore, these medicines are acknowledged for treatment of number of diseases. Various plants derived antioxidant-based therapeutic medicines are being in use for the

prevention and cure of many diseases such as Alzheimer's disease, diabetes, stroke, atherosclerosis, and cancer (Devasagayam *et al.*, 2004). The current study mainly concentrates on the role of MLE in ameliorating the CYP induced oxidative stress in rats. It is evident from the results that MLE ethanol extract possess protective action against liver injuries induced by CYP induced oxidative stress.

Decrease in the level of serum protein, albumin, and globulin was observed after the CYP treatment in rats. The reduction in serum protein, particularly albumin, could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver (Attia and Nasr, 2009). Also, the protein level suppression may be due to loss of protein either by reduce in protein synthesis or increased proteolytic activity or degradation (Yeragi *et al*., 2003). In addition, the observed decrease in serum proteins could be attributed in part to the damaging effect of CYP on liver cells, as confirmed by the increase in activities of serum AST and ALT. Natural antioxidant (MLE) mitigated the changes in the protein by regulating the metabolic activities and protein synthesis.

In the current study, CYP treatment greatly affected hematological parameters. Serum ALT, AST and ALP are sensitive markers employed in the diagnosis of liver damage (Boone *et al*., 2005; Singh *et al*., 2011). The increased activity of plasma ALP, ALT, AST and level of LDH in the CYP–treated rats is a manifestation of induced hepatocellular damage. Increases in the ALP is generally associated with impairment of intrahepatic and extrahepatic bile flow, hepatobiliary injury, erythrocyte destruction or altered bilirubin metabolism (Singh *et al*., 2011; Dufour *et al*., 2000; Burt and Day, 2002). Chemotherapy - induced increase in plasma ALT an AST has been well documented (Ramadori and Cameron, 2010). Increases in ALT and AST activities are indicative of hepatocellular injury or regenerative activity (Meyer and. Harvey, 2004). MLE could have a protector effect against hepatic damage induced by oxidative stress in the CYT state, which was evidenced by the capacity of this polyphenol to modulate the antioxidant defense and to decrease the lipid peroxidation in liver tissues (Schmtz *et al*., 2012).

Results also showed significant increase in serum HDL of animals pretreated with MLE prior to CYP treatment compared to CYP-treated group. HDL may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion. Also, high level of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. Also, HDL could protect LDL against oxidation *in vivo*, because the lipids in HDL are preferentially oxidized before those in LDL (Bowry *et al*., 1992). These alterations in lipid profile may be attributed to the increased lipolysis and fatty acid formation in the blood. Hypertriglyceridemia in combination with abnormally low concentrations of HDL is one of the most common atherogenic profiles of lipid metabolism. Agbor *et al*. (2005) reported that the accumulation of blood triglycerides may be a result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchyma cells into the systemic circulation.

Various studies have confirmed that free radicals are involved in various metabolic alterations and diseases. Radical reactions are mainly responsible for the *in vivo* toxic effects of CYP. Lipids confined in the cell membranes are very sensitive to the oxidative stress. Lipid peroxidation converts polyunsaturated fatty acids into small and more reactive elements. CYP is a toxic chemical which produces various free

radicals causing lipid peroxidation. Increase in lipid peroxidation is calculated in terms of thiobarbituric acid reacting substance (TBARS) which measures the damage caused to the membranes by free radicals (Attia and Nasr, 2009). So, TBARS level content is supposed to be main markers of CYP-induced oxidative stress (Stankiewicz *et al*., 2002). In the current study, decrease in protein content was observed while TBARS level increased due to CYP treatment which was restored to normal level after treating with MLE. During bio activation of CYP, reactive oxygen species are also formed, which can modify the components of both healthy and neoplastic cell leading to decreased antioxidative capacity (Stankiewicz *et al*., 2002). Free radicals produced by bioactivation of CYP increase lipid peroxidation and decrease the level of antioxidant enzymes resulting in damage to liver tissue. MLE restored the damage produced by CYP. On line with the present observation Chattopadhyay *et al.* (2011), found that administration of aqueous extract of *M. oleifera* was found to significantly prevent the arsenic-induced alteration of hepatic function markers and lipid profile.

Various antioxidant enzymes for example, catalase, peroxidase, and superoxide dismutase, play an important role in protecting lung tissues from free radical induced damages (Ganie *et al*., 2011). In the present study, the decrease in level of antioxidant enzymes (CAT) and GSH in liver tissues was observed in CYP treated group. Co-administration of MLE ameliorated the level of antioxidant enzymes, suggesting protective role of antioxidant enzymes against CYP generated free radicals. Similar results were observed by Rakesh and Singh (2010) *M. oleifera* extract restored GSH in rat liver reduced by CCl_4 treatment.

The maintenance of GSH activity in cell is dependent on the level of glutathione reductase and NADH (Meister and Anderson, 1983). Significant decrease in activities of GSH was observed in the present study. The decreased activity of glutathione system in liver tissue of CYP intoxicated rats might be due to the increased lipid peroxidation or inactivation of the antioxidative enzymes. Administration of MLE ameliorated the CYP toxicity, thereby increasing the activity of antioxidant enzymes and GSH. Thus, activation of these antioxidants by the administration of MLE clearly shows that through its free radical scavenging activity could exert a beneficial action against pathophysiological alterations caused by free radicals.

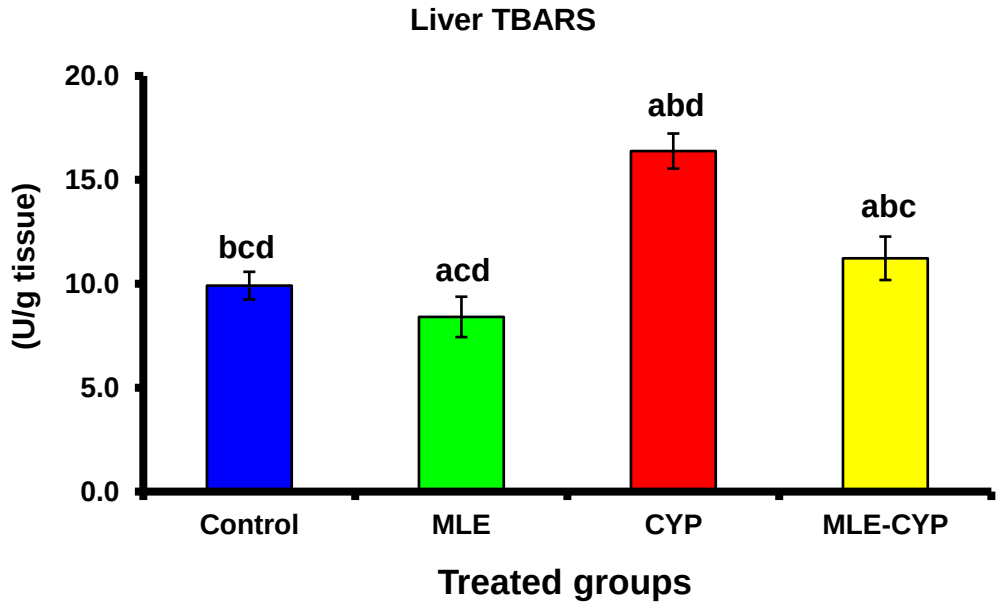

Figure 1: Liver thiobarbituric acid reactive substances (TBARS) (U/g tissue) of rat treated with *Moringa oleifera* leaf extract and cyclophosphamide. MLE: *Moringa oleifera* leaf extract; CYP: Cyclophosphamide; Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of MLE and other groups; [c] Comparison of CYP and other groups; [d] Comparison of MLE-CYP and other groups

Under normal conditions to protect cells against oxidative damage, the potential harmful effects of ROS and free radicals are effectively eliminated by the antioxidant defence systems such as antioxidant enzymes and nonenzymatic factors (Hu *et al*., 2005). Normally, cells possess a well-developed biochemical defence system, comprising low-molecular weight free radical scavengers, i.e. glutathione (GSH), vitamin C, vitamin E and complex enzymes, including GPx, SOD and CAT (Yao *et al*., 2007). When the balance between the generation and elimination of ROS is broken, as a result of these events, biomacromolecules including DNA, membrane lipids and proteins are damaged by ROS-mediated oxidative stress (Qian *et al*., 2008; Ding and Ma, 2018).

Cyclophosphamide decreased liver DNA and RNA concentrations compared to the control. Supporting the present study, Matalon *et al*. (2004) reported that CYP requires microsomal oxidation in the liver to yield its active metabolite, 4-hydroxy-CYP in target cells that spontaneously decomposes to phosphoramid mustard which exerts cytotoxic effect by the induction of DNA single strand breaks as well as crosslinks which result in different types of damage. Also, Bosanquet *et al*. (2002) had reported that CYP involves inhibition of cell division due to cross-linking of the drug to DNA.

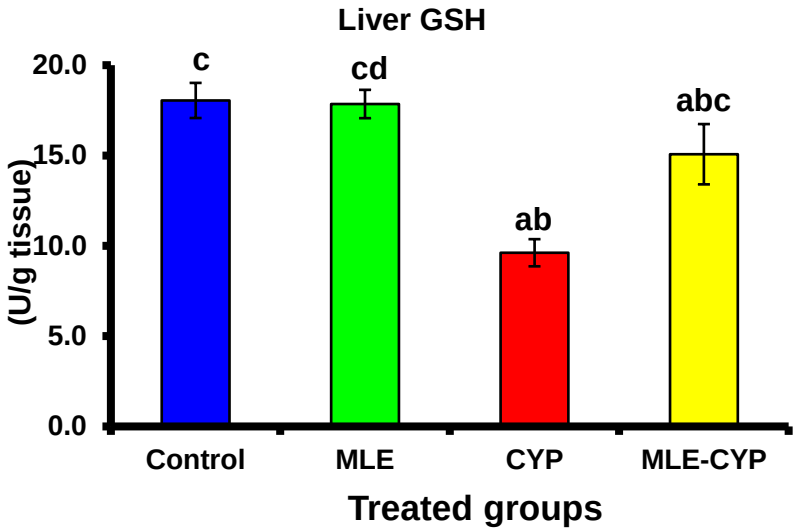

Figure 2: Liver glutathione (U/g tissue) of rat treated with *Moringa oleifera* leaf extract and cyclophosphamide. MLE: *Moringa oleifera* leaf extract; CYP: Cyclophosphamide; Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of MLE and other groups; [c] Comparison of CYP and other groups; [d] Comparison of MLE-CYP and other groups

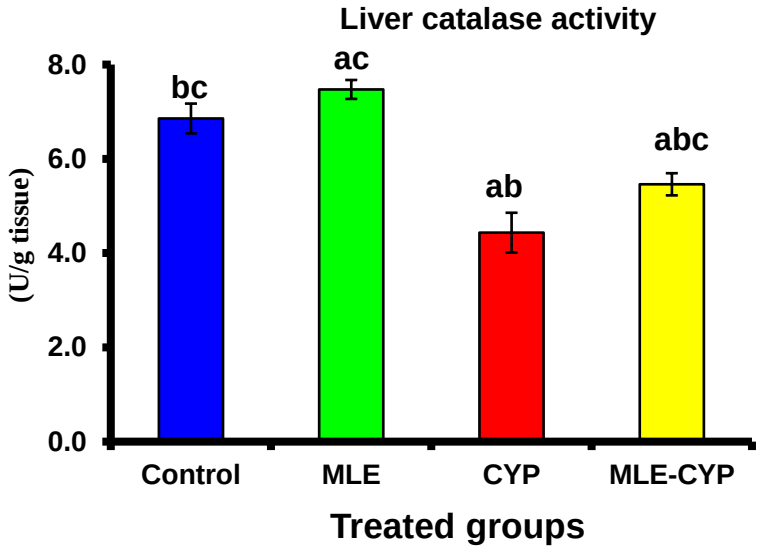

Figure 3: Liver catalase activity (U/g tissue) of rat treated with *Moringa oleifera* leaf extract and cyclophosphamide. MLE: *Moringa oleifera* leaf extract; CYP: Cyclophosphamide; Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of MLE and other groups; [c] Comparison of CYP and other groups; [d] Comparison of MLE-CYP and other groups

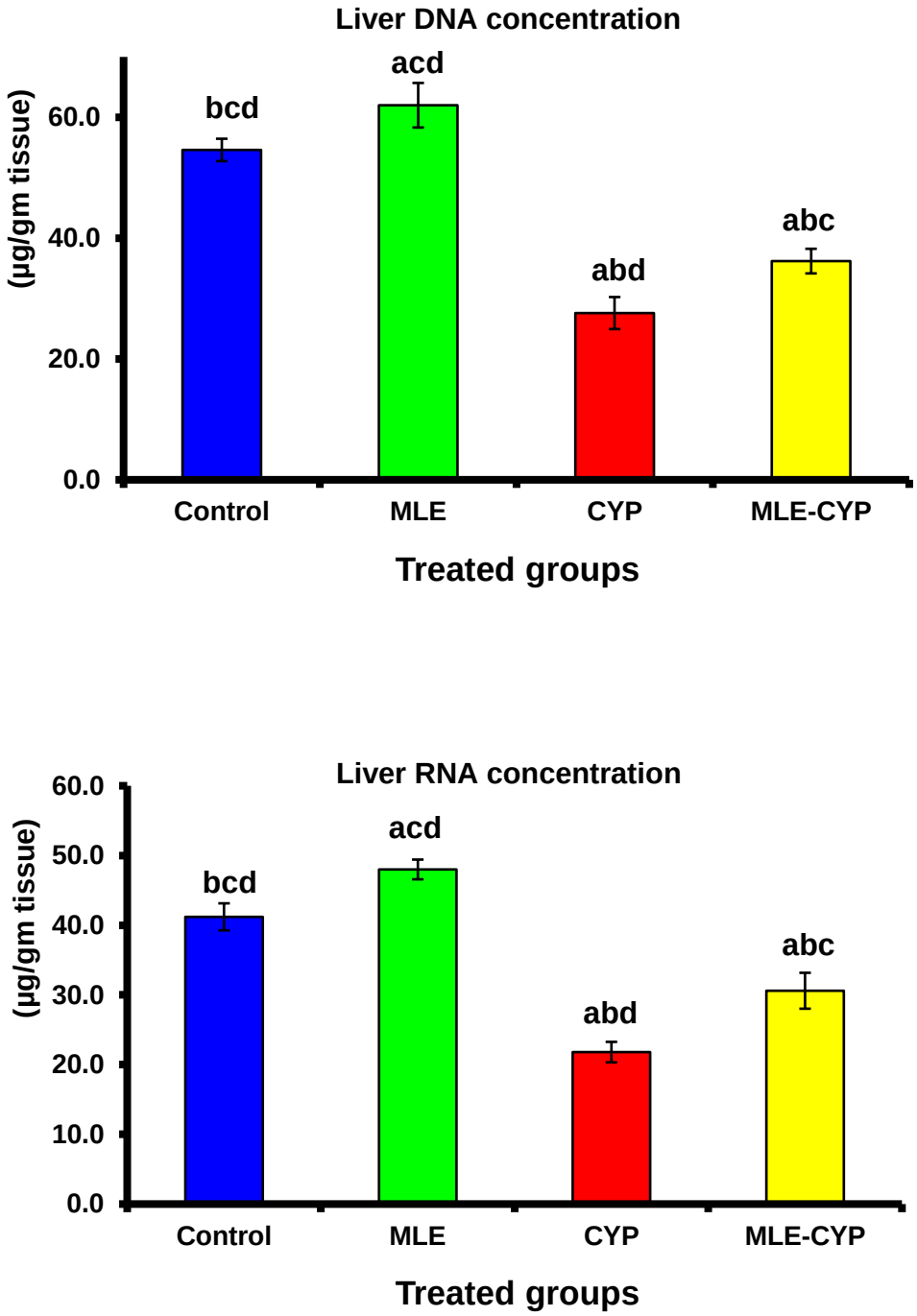

Figure 4: Liver DNA and RNA concentrations (µg/gm tissue) of rat treated with *Moringa oleifera* leaf extract and cyclophosphamide. MLE: *Moringa oleifera* leaf extract; CYP: Cyclophosphamide; Significance at $P < 0.05$. [a] Comparison of control and other groups; [b] Comparison of MLE and other groups; [c] Comparison of CYP and other groups; [d] Comparison of MLE-CYP and other groups

The combination of CYP and MLE was less effective to DNA and RNA compared to individual administration of CYP. In line with the present observation, Ippoushi *et al*., (2003) found a protection against oxidative DNA damage by herbal extracts and formulations. Oxidative damage mediated as single strand breaks in super coiled PTZ18U plasmid DNA has been reported to be suppressed by 6-gingerol (a phenolic compound in ginger). Also, a reduction in the basal levels of oxidative DNA damage upon treatment with 4-coumaric and protocatechuic acids were reported (Guglielmi *et al*., 2003). The results of the present study are also in agreement with the above reports. These findings support the use of the leaves of *Moringa oleifera* to protect against oxidative DNA damage.

5. Conclusions

It can be concluded that Moringa extract contains antioxidant activity as it prevented the oxidative stress and increased the antioxidant effect in liver tissues of male rats. Our results demonstrate the CYP protective role against CYP generated free radicals damages and suggest for further study to isolate the bioactive component in pure form from MLE.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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