Argan oil (*Argania spinosa L*) Provides protection against mercuric chloride induced oxidative stress in rat *Albinos Wistar*

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Abstract

The present study was undertaken; to evaluate the protective effect of argan oil against mercuric chloride induced oxidative stress in experimental rats. Adult male albinos wistar rats randomly divided into four groups, were the first was served as a control, whereas the remaining groups respectively treated with: argan oil (5ml/ kg b.w; by gavage), mercuric chloride (0.25 mg/kg body weight i.p) and combination of argan oil and $HgCl_2$. Change in liver enzyme activities, thiobarbituric acid reactive substances (TBARS) level, antioxidants and reduced glutathione (GSH) contents were determined after 3weeks experimental period. Exposure of rats to mercuric chloride caused a significant increase the lipid peroxidation level along with corresponding decrease in the reduced glutathione and various antioxidant enzymes in liver. And increase in serum: total billirubin, direct billirubin levels, APL, LDH and transaminases activities. Supplementation of argan oil resulted in decreased of lipid peroxidation level and in the serum: AST, ALT, APL and LDH activities were decreased along with increase in liver GSH level. The activities of antioxidants enzymes: glutathione peroxidase (GSH-Px) and glutathione –S-transferase (GST) were also concomitantly restored to near normal level by argan oil supplementation to mercuric chloride intoxicated rats. Liver histological studies have confirmed the changes observed in biochemical parameters and proved the beneficial role of argan oil. The results clearly demonstrate that argan oil treatment augments the antioxidants defense mechanism in mercuric chloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases.

Keywords: Antioxidant enzymes, Argan oil, Mercury, Oxidative stress, Rat.

1   Introduction

Mercury a highly toxic metal, results in avariety of adverse health effects including neurological, renal, respiratory, immune, dermatologic, reproductive and developmental sequelle [1].Due to wide use of mercury in agriculture, industrial, medical and other fields, its exposure is cannot be avoided. The toxicity the mercury compounds (elemental, inorganic and organic). Inorganic mercury present in the environment is a well- established toxicant to human health [2]. Exposure to mercury promotes the reactive oxygen species (ROS) formation such as hydrogen peroxides, these ROS enhances the subsequent iron and copper induced production of lipid peroxides and highly reactive hydroxyl radicals [3, 4]. Detrimental effects caused by free radicals occur when there is an imbalance between free radical production and radical scavenging capacity of   antioxidant system in favour of former [5]. Mercury induced oxidative stress; make an important contribution to molecular mechanism for liver injury [6]. Recent evidences also show that mercury causes severe oxidative damage [7], thus mercury is proved to be a potential oxidant in the category of environmental factors. Therefore, there is a need to provide protection against mercury induced toxicity. It is important to develop an effective drug for mercury to prevent the mercury induced cellular damages. Historically, plants have been used as folk medicine against various type of disease.
Argan oil obtained from *Argania spinosaL*. This oil is also used in traditional medicine [8]. Chemical analysis of this oil highlighted a glyceride fraction (99%) that is mainly rich in polyunsaturated fatty acids like oleic (47.7%) and linoleic (29.3%) [9]. Studies with the unsaponifiable fraction revealed, that argan oil is rich in tocopherol (62mg/100g verus 320mg/kg in olive oil and 400mg/kg in sunflower oil), particulary α and γ –tocopherol [10,11]. This fraction also contains other important compounds such as squalene, sterols (schottenol and spinasterol) and phenols (ferulic, syringic

and vanillic acid) [10]. These compounds make argan oil an important source of antioxidant [9], which certainly play an important role in vivo. This compound makes argan oil a very important source of vitamin E and also contribute to better preservation of this oil since it protects against oxidation [12]. However, its antioxidative potential against mercury induced oxidative stress remains unexplored.

Therefore, the purpose of this study was to delineate the beneficial effects of argan oil against oxidative stress induced by mercuric chloride in rats.

2   Materials and methods

The argan oil used in this work originated from Tindouf (south-west of Algeria). It was extracted by a traditional method.

All chemicals used in this work were purchased from sigma chemical company. Laboratory animals, albino wistar male rats, were brought from the Algiers Pasteur institute at the age of 8 weeks, with an average live weight of 200g. They were located in a room with an ambient temperature of $21 \pm 1°C$ and up to 12h of light daily. The rats were divided into four experimental groups; each consists of eight rats. The first group was served as the control. The second group was given argan oil at a dose of 5ml/kg body weight, while the third group ($HgCl_2$) was intraperitoneally given mercuric chloride at a dose of 0.25 mg/kg body weight. Finally, the fourth group: argan oil orally was given (5ml/kg body weight) 10 days before $HgCl_2$ (0.25 mg/kg body weight) and continued up to 3weeks after mercuric chloride treatment. The treatment of all groups was lasted for 3consecutive weeks.

Twenty four hour after the last administration the blood was collected by retro- orbital sinus punction from each anesthetized rats. After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at−20°c until determination of: billirubin levels and enzymes (AST, ALT, ALP and LDH) activities. Subsequently, rats were decapitated and liver were removed.

2.1   Tissue preparation

About 500mg of liver was homogenized in 4ml of buffer solution of phosphate buffered saline (w/v: 500mg tissue with 4ml PBS, PH 7.4) homogenates were centrifuged at 10.000xg for 15min at 4°c. The resultant supernatant was used for determination of: reduced GSH, Thiobarbituric acid- reactive substance (TBARS) levels, and the activities of: GSH-PX and GST.

2.2   Determination of billirubin levels and enzymes

Serum billirubin levels and AST, ALT, ALP and LDH activities were determined spectrophotometrically using an automated analyzer.

2.3   Determination of lipid peroxidation (LPO)

Lipid peroxidation level in the liver was measured by the method of Buege and Aust (1978) [13]. 125µl of supernatant were homogenized by sonication with 50 µl of PBS, 125 µl of 20% TCA + BHT 1% (TCA-BHT) in order to precipitate proteins, and centrifuged (1000xg, 10min, 4°c), afterwards, 200µl of supernatant were mixed with 40µl of HCl (0,6M), and 160µl of TBA dissolved in tris (120 mM). And the mixture was heated at 80°c for 10min; the absorbance was measured at 530nm. The amount of TBARS was calculated by using a molar extinction coeffient of 1.56×10^{5} M/Cm.

2.4   Determination of reduced glutathione (GSH)

GSH content in liver was measured spectrophotometrically by using Ellman's reagent (DTNB) as a colouring reagent, following the method described by Weeckbeker et cory (1988) [14].

2.5   Determination of glutathione-S-transferase (GST) (EC2.5.1.18)

The cytosolic glutathione-S-transferase activity was determined spectrophotometrically at 37°c by method of Habig et al (1974) [15]. The reaction mixture (1ml) contained 0.334ml of 100mM phosphate buffer (PH 6.5), 0.033ml of 30mM CDNB and 0.33ml of reduced Glutathione. After pre-incubating for 2min, the reaction was started by adding 0.01ml of diluted cytosol and the absorbance was followed for 3min at 340 nm. The specific activity of GST is expressed as µmole of GSH-CDNB conjugate formed/ min /mg protein using extinction coefficient of 9.6 Mm^{-1} cm^{-1}

2.6 Determination of GSH-Px (E.C.1.11.1.9)

Glutathione peroxidase (EC 1.11.1.9) activity was modified from the method of Flohe and Gunzler (1984) [16]. for the enzyme reaction , 0.2ml of the supernatant was placed into a tube and mixed with 0.4ml GSH (reduced glutathione, sigma product, analytical grade), and the mixture was put into an ice bath for 30min. Then the mixture was centrifuged for 10min at 3000rpm, 0.48ml of the supernatant was placed into a cuvette, and 2.2ml of 0.32M Na_2HPO_4 and 0.32ml of 1m mol/l 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, sigma) were added for color development. The absorbance at wavelength 412nm was measured with a UV spectrophotometer after 5min. The enzyme activity was calculated as a decrease in GSH within the reaction time as compared with the non-enzyme reaction.

2.7 Protein quantification

Protein was measured by the method of Bradford (1976) [17], using bovine serum albumin as the standard.

2.8 Histopathological examination

Liver from autopsied animals were excised out and fixed in formalin (10%). five micron think section were prepared by using microtome and these section were stained with hematoxyline and eosin. For histological alterations these slides were observed under light microscope.

2.9 Statistical analysis

The data were subjected to student t test for comparison between groups. The values are expressed as mean $\pm$ SEM. Significance level was set at $P<0.05$, $P<0.01$, $P<0.001$.

3   Main results

3.1 Effects of treatments on serum biochemical parameters

Treatment with $HgCl_2$ caused a significant ($P \leq 0.01$) increase in the activities of AST, ALT, ALP and LDH as compared to mercuric chloride treated rats. Only argan oil treatment did not show any significant alteration. However, the combined treatment of argan oil with mercuric chloride results in gradual recovery in AST, ALT, ALP and LDH activities as compared to mercuric chloride treated rats(table 1). A highly significant ($P \leq 0.001$) elevation in serum total billirubin and direct billirubin was observed in mercuric chloride intoxicated rats. Only argan oil treatment did not show any significant alteration. However, the combined treatment of argan oil with mercuric chloride results in Gradual recovery in total billirubin and direct bilirubin levels was noticed respect to mercuric chloride treated animals (table1).

3.2 Effects of treatments on hepatic oxidative stress parameters

Mercuric chloride exposure a highly significant depleted in reduced glutathione (GSH) level, GPx and GST activities. And a significant increase in liver lipid peroxidation level in mercury intoxicated rats was noticed. Argan oil alone treatment did not show any significant decline. In combined treatment of argan oil with mercuric chloride a highly significant increase in reduced glutathione (GSH) level, GPx and GST activities. And a highly significant depletion in lipid peroxidation level was recorded with respect to mercury intoxicated rats (Figs. 1 and 2).

3.3 Histological studies

Mercuric chloride induces various pathological alterations in liver of rats. These   alterations   were characterized by centrilobular necrosis, degranulation, destruction of membrane cells, cytoplasmic vacuolization (Fig.

Table 1: Changes in biochemical parameters of control and rats treated with organ oil, mercuric chloride, and combined treatment of mercuric chloride with argan oil after 3weeks of treatment.

Parameters	experimental groups			
	control	argan oil	HgCl$_2$	argan oil + HgCl$_2$
SGOT(U/L)	126,45 ± 62.4	110.91 ± 63.9	215.5 ± 66.6 **	132.45 ± 37.3***
SGPT(U/L)	48.38 ± 9.28	49.7 ± 9.31	83.39 ± 3.05***	50.69 ± 11.1***
ALP(U/L)	201.3 ± 79.8	203.0 ± 40.7	571.18 ± 126***	211.58 ± 58.4***
LDH(U/L)	409.25 ± 208	374.63 ± 101	594 ± 118 *	456.53 ± 46.7***
Total Bllirubin(mg/dl)	1.84 ± 0.15	1.73 ± 0.35	3.59 ± 0.35 ***	1.9 ± 0.09***
Direct Billirubin (mg/dl)	0.35 ± 0.11	0.28 ± 0.09	0.73 ± 0.03 ***	0.37 ± 0.11***

Values are given as mean ± SEM for group of 8 animals each. *P≤0.05, compared to controls. **P≤0.01, compared to controls. ***P≤0.001, compared to controls.
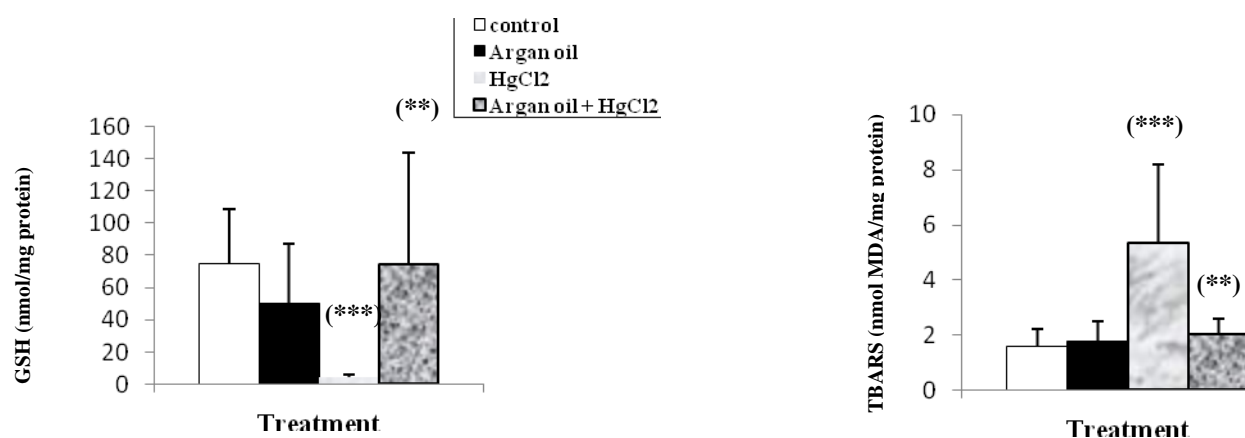

Fig.1. Reduced glutathione (nmol/ mg protein) and TBARS (nmol MDA /mg protein) levels in liver of control and rats treated with argan oil, mercuric chloride and combination of argan oil and mercuric chloride after 3weeks of treatment. Values are given as mean ± SEM for group of 8 animals each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).
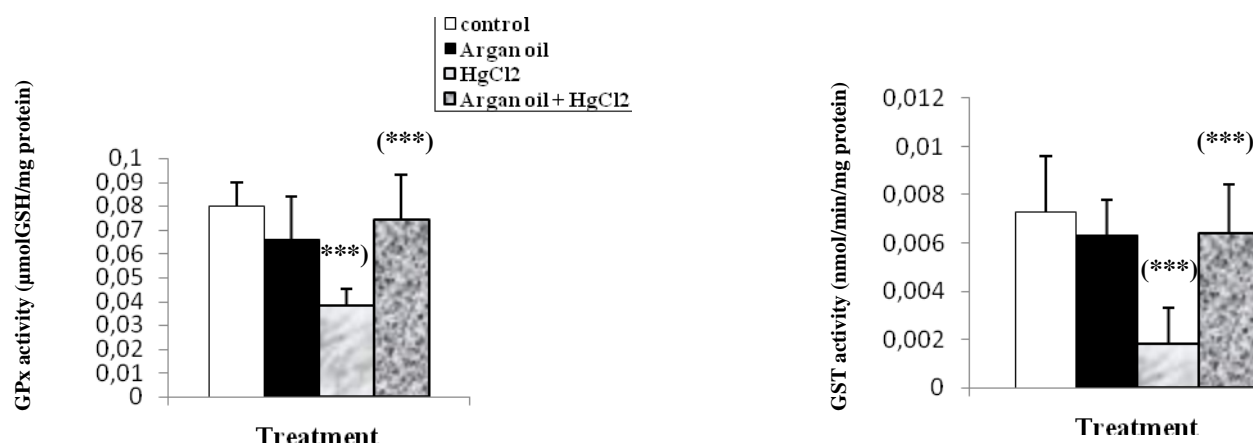

Fig.2. Enzyme activities of GPx (µmol GSH/ mg protein) and GST (nmol /min/mg protein) in liver of control and rats treated with argan oil, mercuric chloride and combination of argan oil and mercuric chloride after 3weeks of treatment. Values are given as mean ± SEM for group of 8 animals each significant difference: * compared to controls (*P≤0.05; **P≤0.01; ***P≤0.001).

3C). In combination group were argan oil was administered with mercuric chloride showed reparative changes. Liver showed prominent recovery in the form of normal hepatocytes and very less centrilobular necrosis. Pronounced sinusoid .With granular hepatocytoplasm were also evident (Fig.3D). Liver of the control group had a regular histological structure with a characteristic pattern of hexagonal lobules (Fig. 3A).Furthermore, no histological alterations were observed in the liver of argan oil treated group (Fig. 3B).
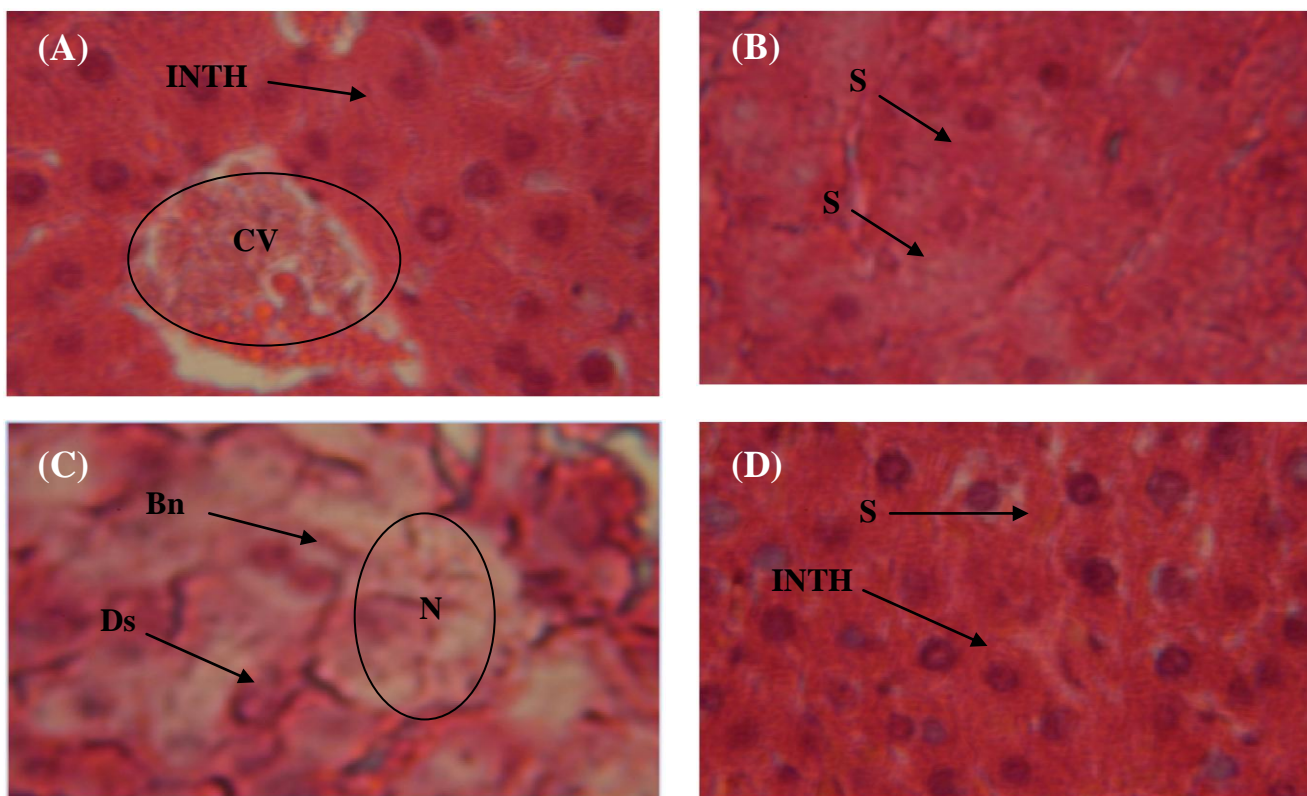

Fig.3. Microscop evaluation of hepatic tissue from (A) control, (B) treated with argan oil, (C) mercuric chloride and (D) argan oil pre- and post treated with mercuric chloride after 3weeks of treatment, section were stained using the hematoxylin-eosin method (400X). Bn: Bright nuclei, Ds: destruction of membrane cells, N: Necrosis, INTH: Intact hypatocyte cells, CV: central vein, S: Sinusoid. Argan oil coadministrated with mercuric chloride shows granular cytoplasm and normal hepatocytes.

4   Discussion

The present investigation revealed that mercuric chloride intoxication causes significant increase in lipid peroxidation, total billirubin and direct billurubin levels, AST, ALT, ALP and LDH activities, and significant decrease in reduced glutathione, glutathione peroxidaseand glutathione-s-transferase in liver.

The principal toxic effects of mercury involve interaction with a large number of cellular processes, including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative stress [18]. Once absorbed in the cell, both Hg^{+2} and MeHg from covalent bonds with GSH and the cysteine residue of proteins. GSH, the primary intracellular antioxidant and the conjugating agent, was shown to be depleted and to have impaired function in Hg toxicity. A single Hg ion can bind to and cause irreversible excretion of up to two GSH molecules [19]. In fact, GSH serves as a primary line of cellular defense against Hg compounds. Released Hg ions form complexes with GSH and cysteine results in greater activity of the free Hg ions, disturbing GSH metabolism and damaging cells [20]. As a result of binding of mercury to glutathione, levels of GSH are lowered in the cell and decrease the anti-oxidant potential of the cell. Antioxidant enzymes such as glutathione peroxidase and glutathione –S-transferase play a major role in the intracellular defense against oxygen radical damage to aerobic cells. Chung et al (1982) [21] demonstrated that 10mg/kg of mercury caused time dependent decreases in the activities of the enzyme of the glutathione metabolism pathway in the rat kidney. Girardi and Elias (1995) [22] reported that mercury inhibits the activities of redox cycles

enzymes. Recent finding of Bando et al (2005) and jadhav et al (2006) [23,24] proved our point that this antioxidant enzymes show decreased level following mercury intoxication. Because of the low activity of antioxidant enzymes in the liver and decreased content of GSH, the liver is hypothesized to be highly susceptible to oxidative stress. Mercury induced oxidative stress turn to severe; the inbuilt mechanism of body fails to alleviate the damage. It has been demonstrated that mercury decreases the anti-oxidative systems and produces oxidative damages via H_2O_2 generation therby leading to lipid peroxidation [25,26]. All these possible mechanisms of mercuric chloride toxicity may lead to the formation of reactive oxygen species (ROS), as found in the present investigation. Therefore, an increase in the formation of ROS by mercuric chloride may induce membrane biochemical and functional alterations and thus induced liver cell damage.

Further, mercury intoxication also induces a significant elevation in serum billirubin levels and: AST, ALT, ALP and LDH activities. This increase may be due to cellular necrosis of hepatocytes, which causes increase in the permeability of cell resulting release of transaminases and ALP, LDH and billirubin in blood stream [27-29]. This confirms our earlier reports on histopathological alterations in liver induced by mercury intoxication [29,30].

It was observed that argan oil when given in combination with mercuric chloride significantly increases liver GSH level, GSH-Px and GST activities as antioxidant potential and thereby declines the level of lipid peroxidation, which in turn reduces the transaminases, ALP and LDH activities and billirubin level in serum. In present investigation, the elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and directly detoxifies reactive species [31]. Glutathione, as both a carrier of mercury and an antioxidant, has specific roles in protecting the body from mercury toxicity. Glutathione, specifically bind with methylmercury, forms a complex that prevents mercury from binding to cellular proteins and causing damage to both enzymes and tissue [32]. Glutathione-mercury complexes also reduce intracellular damage by preventing mercury from entering tissue and cells, and becoming an intracellular toxin. The elevated level of GSH-Px and GST by argan oil as compared to the $HgCl_2$ may have facilited the conjugation reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophile for inactivation of electrophiles and therefore might be playing a major role in metalloprotection.

Some of the active constituents of argan oil have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system. Antioxidants are compounds that can delay or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction [33].The protective effect of argan oil is probably due to its high contents of powerful antioxidants, particularly: polyunsaturated and unsaturated fatty acid, polyphenols, tocopherols, sterols and β -carotene, which are known as powerful antioxidants [34]. This products act by several mechanism: scavenging of peroxy radicals, which break the peroxidation chain reaction, chelating free cu^{+2} to form redox-inactive complexes and thus reducing metal-catalyzed oxidation of lipid and inhibiting the binding of cu^{+2} to apolipoproteins and subsequently preventing the modification of amino acid-Apo-B protein residue [35,36]. polyunsaturated and unsaturated fatty acid have protective effects against oxidative stress induced by mercuric chloride in rats, which are explained by their double bounds, that is difficult to oxidize and that involved in the fluidity of lipoproteins [37]. The antioxidant activity of polyphenolics is principally difined by the presence of orthodihydroxy substituents, which stabilize radicals and chelate. The antioxidant effect of phenolic acids and their esters depends on the number of hydroxyl group in the molecule. Argan oil, with comparison to olive oil, contains a higher quantity of ferulic acid (3470±13 verus 51±2 μg/kg of oil, respectively) [10]. This acid is more effective than ascorbic acid and other phenolic acid such as p-coumaric acid, since the electron donating methoxy group allows increased stalization of the resulting aryloxyl radical through electron delocalization after hydrogen donation by the hydroxyl group [38]. This showed that, the direct inhibition of trans-conjugated dienehydroperoxyde isomer formation is related to the H-donating ability of the phenol [39]. Argan oil, but not olive or sunflower oil, also contains another important phenolic acid: syringic acid (68±4μg/kg), this antioxidant compound protects against lipid peroxidation [40].

β -carotene of argan oil may scavenger free radicals generated by mercuric chloride and reduces the lipid peroxidation. The antioxidant mechanism of β -carotene has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation [41,42].

Vitamin E, function as a trap for lipid peroxyl (LOO) and other radicals, effectively inhibiting the peroxidation of cellular membranes.vitaminE prevents lipid peroxidation and maintains GSH and ascorbic acid levels in damaged tissue by inhibiting free radical formation [43]. According to Roe and Sharma (2001) [44], vitamin E showed protective effect against $HgCl_2$ may be due to impaired absorption of mercury in the gastrointestinal tract. Ran et al (1996) [28] postulated that, vitamin E has a protective effect against mercury toxicity. Vitamin E inhibits oxidative damage caused by mercury and cadmium intoxication [28,39]. Saponin content of argan oil significantly inhibited peroxyl radical induced lipid peroxidation in rat liver.

It may be concluded that combined treatment of argan oil has a preventive and protective effect on mercuric chloride induced oxidative stress. More-over, it protects from $HgCl_2$ induced hepatic dysfunction and executes its modulatory role in mercury induced free radical production.

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