

Hepatoprotective Effects of Moringa Oleifera Seeds Against Ethanol Induced Liver Damage In Wistar Rats

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Abstract

Hepatocytes are reportedly susceptible to the injurious effects of oxidants when exposed to toxic substances such as Ethanol . The widespread claims of the medicinal efficacy of various parts of Moringa oleifera plant have been well documented in literature. Oil was extracted from seeds and acetone extract was prepared from defatted seeds and evaluate antioxidant properties. The in vitro antioxidant of oil and acetone extract of Moringa oleifera (M. oleifera) seeds were assayed by DPPH scavenging activity and reducing power. The in vivo hepatoprotective effects evaluated in male Wistar rats against ethanol induced liver damage in preventive and curative models. The M. oleifera oil and acetone extract (300 mg/kg body weight (bw), and silymarin (100 mg/kg bw) were administered orally in both the studies. Liver injury was induced by 40% ethanol administration (3.76 gm/kg bw, orally) for 30 days. Both moringa oil and acetone extract showed a good amount of phenols and flavonoids and appeared antioxidant activity in reducing power and DPPH scavenging activity assay, while the oil of moringa was the more effective one compared with acetone extract. The level of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) , total bilirubin , albumin, total protein and plasma antioxidant state (MDA content and catalase activity) were determined to assay hepatotoxicity. Ethanol administration caused severe hepatic damage in rats as evidenced by elevated plasma AST, ALT, ALP , total bilirubin, albumin, , total protein , MDA content and catalase activity levels. The oil , acetone extract of M. oleifera and silymarin administration prevented the toxic effect of ethanol on the above plasma parameters in preventive model. The present study concludes that oil and acetone extract of M. oleifera seeds have significant antioxidant and hepatoprotective activity against ethanol induced hepatotoxicity.

Key words: Moringa oil , Moringa acetone extract, ethanol, antioxidant, liver,.

1- Introduction :

Throughout the world, plants have been a rich source of nutrients and antioxidants as they contain lot of bioactive molecules and compounds. Out of the bioactive molecules, most of them are as produced as chemical defense against stresses or infections. Native plants usage in traditional as well as modern medicine is gaining a lot of attention now a days, and the recent studies showed that a number of plant products and herb extracts exert potent antioxidant actions (Karthishwaran and Mirunalini 2012). Liver is the largest organ of the human body weighing approximately 1500g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm (Singh et. al., 2011). The liver is a highly sensitive organ which plays a major role in maintenance and performance of the homeostasis in our body. It is the chief organ where important processes like metabolism and detoxification take place. Thus the liver is prone to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotics (Amacher 2002) . The liver disorders are one of the serious health problems, throughout the world. More than 350 million people were affected with chronic hepatic infections and in India above 20,000 deaths were reported every year due to liver disorders. Hepatocellular carcinoma is one of the most common tumors in the world with over 250,000 new cases each year (Salha and Canelo 2011). Hepatotoxicity can be characterized into two main groups, each with a different mechanism of injury: hepatocellular and cholestatic (Navarro and Senior 2006) . A hepatocellular pattern is marked by isolated or predominant elevations of serum transaminases (Musana et. al., 2004). On the other hand, cholestatic injury is characterized by predominantly initial alkaline phosphatase level elevations that precede or are relatively more prominent than increases in the levels serum transaminases (Singh

et. al., 2011). Generally mixed type of injuries, involving both hepatocellular and cholestatic mechanisms, occurs (Teschke 2009). Chemical agents that cause liver injury are otherwise called hepatotoxins. Ethanol is a lipid-soluble non-electrolyte, which is readily absorbed from the skin and gastrointestinal tract, diffuses briskly into circulation and dispersed evenly all the way through the body (McDonough 2003). The greater part of ethanol is metabolized in the liver and individuals who get addicted to alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for budding alcoholic liver disease (Zakhari and Li 2007). In addition, both acute and chronic administration of ethanol causes formation of cytokines in large amounts, particularly TNF- α by hepatic Kupffer cells, which play a chief role in causing liver injury (Thurman 1998), (Tsukamoto et. al. 2001) and (Zhou et. al. 2003). Moreover, chronic administration of ethanol results in accumulation of hepatic lipids as well as lipid peroxides which lead to autooxidation of hepatic cells either by acting as a pro-oxidant or by decreasing the antioxidant levels, thereby resulting in a noteworthy hepatotoxicity (Crawford and Blankenhorn 1991). Lipid peroxidation by ethanol induces hepatic oxidative stress which has been identified to take part in a pathogenic role in Alcoholic Liver Disease (ALD) (Bunout 1999). Liver injury can be diagnosed by certain biochemical markers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (Singh et. al. 2011). The elevation of serum activities of hepatocellular enzymes ALT, AST and ALP is a reflection of their increased passage into the serum from damaged liver cells (Olatosin et. al. 2013). Serum alkaline phosphatase increases to some extent in most types of liver injury (Sturgill and Lambert 1997). Bile acids account for this increase: they induce alkaline phosphatase synthesis and exert a detergent effect on the canalicular membrane, allowing leakage into serum (Kaplan 1986, 1993). The highest concentrations are observed with cholestatic injuries (Friedman et. al. 1996). At present, the most commonly used antioxidants are BHA, BHT, propyl gallate and tert butylhydroquinone. Besides this BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Sherwin 1990). Therefore, there is a growing interest on natural and safer antioxidants (Moure et. al. 2001). In recent days, the use of herbal natural product has increased attention among the world population. Many of the herbal supplements are claimed to assist in healthy lifestyle. Among those herbs, is *Moringa oleifera* Lam (MO) (Family: Moringaceae), commonly known as drumstick tree or horseradish tree. Drumstick has been claimed in traditional literature to be valuable against a wide variety of diseases. Indian Materia Medica describes the use of roots of *M. oleifera* in the treatment of a number of ailments, including asthma, gout, lumbago, rheumatism, enlarged spleen or liver and internal deep seated inflammations (Fuglie 1999). In recent decades, the extracts of leaves, seeds and roots of *M. oleifera* have been extensively studied for many potential uses including anti-tumour (Guevara et. al. 1999), hepatoprotective (Al-Said et. al. 2012), and antioxidant (Santos et. al. 2012). Keeping these folkloric claims and reports in view, the present study attempted to assess the possible hepatoprotective potential of the oil and acetone seed extract of *M. oleifera* in ethanol-induced hepatotoxicity in rats.

2- MATERIALS AND METHODS:

Chemicals: Folin-Ciocalteu phenol reagent, 1,1- diphenyl-2-picrylhydrazyl (DPPH), gallic acid, potassium ferricyanide, trichloroacetic acid, potassium hydroxide were purchased from Sigma Chemical Company (Germany). Ascorbic acid, solvents and all other chemicals were of analytical grade from BDH Chemical Laboratory (England, UK).

Plant seeds collection:

The seeds of *M. oleifera* were obtained from the National Research Centre, Dokki, Cairo, EGYPT. They were identified and authenticated at the Department of Botany, Faculty of Agriculture, University of Menoufia.

Oil extraction from *M. oleifera* seeds:

M. oleifera whole seeds (100 g) were dehusked and crushed with mortar and pestle. Oil was extracted from the ground seeds with petroleum ether (0.5 L) for 72 h within agitation. The solvent of extraction was evaporated over water bath for 12 h or until completely evaporated.

Extract preparation:

The defatted *Moringa* seeds were extracted at room temperature, defatted seeds were soaked in 700 ml/L aqueous acetone (1 L) for 5-7 days. The soaked material was stirred every 18 h using a sterilized glass rod. The final extract was passed through Whatman filter paper No.1. The filtrate obtained was concentrated under vacuum on a rotary evaporator at 40°C and stored at 4°C for further use.

Identification of fatty acids:

Saturated, unsaturated and total fatty acids were determined in the oil by using methyl esters boron trifluoride method (A.O.A.C 2012) , the oil is saponified with sodium hydroxide in methanol .the fatty acids are methylated with boron tri fluoride in methanol, extracted with heptanes and determined on a gas chromatograph with FID detector (PE auto system XL) with auto sampler and Ezchrom integration system . carrier gas (He), ca.25 Psi –air 450 ml/min –Hydrogen 45 ml – split 10 ml/min . oven temperature 200 C injector and detector 250 C.

Identification of *Moringa oleifera* acetone extract :

GC / MS analysis : the analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass – selective detector (MSD,Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane)capillary column (30 m×0.25 mm i.d. and 0.25 um film thickness) the carrier gas was helium with the linear velocity of ml/min . The identification of components was based on comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Estimation of total phenol content:

The total phenol content of the oil and extract samples were determined using Folin- Ciocalteu reagent according modified method of (Khanahmadi et al. 2010). A 1 mL aliquot of samples ($100 \mu\text{g mL}^{-1}$) was put in test tube, 2.5 mL Folin-Ciocalteu reagent (0.2 M) and 2 mL sodium carbonate (7.5%) was added, allowed to stand in the dark for 20 min at room temperature, thereafter the absorbance was read at 765 nm. The amount of total phenolic component in the oil and extract samples were determined from gallic acid calibration curve and expressed as mg of Gallic Acid Equivalent per gram sample (mg GAE g^{-1}).

Estimation of total flavonoid content:

Total flavonoid of the oil and extract samples were determined by aluminum trichloride colorimetric method using rutin as standard (Nile and Khobragade 2010). The method was based on formation of a flavonoid-aluminum complex. The sample (0.1 mL) in methanol ($100 \mu\text{g mL}^{-1}$) was mixed with 0.2 mL of 5% sodium nitrate, then allowed to react for 5 min thereafter 0.2 mL aluminum trichloride in methanol (10%) and 1 mL of sodium hydroxide (1 M) were added, then allowed to stand at room temperature for 15 min. The absorbance was read at 510 nm against reagent blank. The amount of flavonoid was calculated from rutin calibration curve, results expressed as mg of Rutin Equivalent per gram of sample (mg RE g^{-1}).

Determination of reducing power:

Reducing power ability of the samples were determined using the method of (Adesegun et al. 2008) by mixing 2.5 mL of oil and extract samples of various concentrations (25, 50, 75, $100 \mu\text{g mL}^{-1}$), with 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added and centrifuged ($1000 \times g$, 10 min). The supernatant (2.5 mL) was mixed with equal volume of distilled water and ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a reagent blank.

2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The antioxidant activity of the oil , extract and the standard were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method (Braca et. al. 2002). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 0.004% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Methanol (1 ml) with DPPH solution (0.004%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

$$\text{Percent (\%) inhibition of DPPH activity} = A - B / A$$

Where A = optical density of the blank and B = optical density of the sample.

In vivo study for test the protective effect of Moringa seed oil and acetone extract against ethanol administration:

Animal experiments:

Rats were obtained from Research Institute of Ophthalmology, Giza, Egypt. And the work was carried out at its animal house. To study the protective effect of moringa seed oil and acetone extract oral administration in albino rats; twenty five male albino rats (weighing between 90 and 110 g) were used for this investigation. The rats were fed ad libitum on a basal diet (BD) and water for 15 days as an adaptation period. There were housed individually in stainless steel cages and divided into five groups of five. All groups were fed the BD. Diet intake was monitored daily. The first group (C) was used as controls and received tap water as drinking water. The other four groups; received tap water and ethanol (40% V/V) at a dose of (3.76 g/Kg body weight) by stomach tube, daily for 30 days. The second group (ethanol group) doesn't have any other treatment, the third group (ethanol + moringa seed oil group) was treated simultaneously by stomach tube with moringa seed oil (300 mg/Kg body weight), the fourth group (ethanol + moringa acetone extract group) treated with moringa acetone extract (300 mg/Kg body weight), while the last group (ethanol + silymarin group) treated with silymarin drug (100 mg/Kg body weight). All rats fasted before blood sampling. The blood samples were drawn from eye plexuses, after 30 days, the rats were anesthetized using diethyl ether.

Blood sampling and analysis:

Blood samples were collected after 30 days in tubes contain heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. to obtain plasma, which was kept frozen until analysis. The both of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities were measured according to the method described by (Retiman and Frankel 1957). Alkaline phosphatase (ALP) activity was measured by the method of (Hausamen et al., 1967). Total bilirubin was determined according to (Walter and Gerade 1970). The content of malondialdehyde (MDA) was determined spectrophotometrically at wave length 532 nm according to the method of (Draper and Hadley 1990). Catalase (CAT) activity was determined at wave length 510 nm according to the method described by (Beers and Sizer 1952). Urea was determined according to (Weatherbum 1987), creatinine was determined according to (Kostir and Sonka 1952), and uric acid was determined according to the method described by (Fossati et al., 1980).

Statistical analysis:

The results of the animal experiments were expressed as the Mean \pm SD and they were analyzed statistically using the one-way analysis of variance ANOVA followed by compare means with Duncan's multiple range test. In all cases $p \leq 0.05$ was used as the criterion of statistical significance.

3- Results:

Fatty acids composition:

As shown in Table (1) Moringa seed oil was found to contain a high level of oleic acid (C18:1 n-9), which accounted for (66.12 %) of the total fatty acid. Thus, Moringa oil belongs to the oleic acid oil category (Sonntag 1982). The presence of polyunsaturated (PUFAs, 1.03 %) and saturated fatty acids (SFs, 21.73 %) were very low in comparison with the monounsaturated fatty acids (MUFAs, 76.51 %). Thus, Moringa oil presented relatively low contents of saturated fatty acids (SFAs) and high contents of unsaturated fatty acids (UNFAs) compared with other common vegetable/fruit seed oils, such as corn, olive and sesame and soybean (Manzoor, et al. 2007). High oleic acid in moringa oil makes it desirable in the term of nutrition and high stability cooking and frying oil (Abulkarim, et al. 2005). The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rate of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, Moringa oils with high proportion of oleic acid are more stable than the others. Also, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the n-6 series (linoleic acid). Another interesting fact is that considerable content of linoleic acid (C18:2) as an essential fatty acid in the Moringa oil may provide high nutritional remuneration and render beneficial healthy effect on blood lipid, blood pressure and cholesterol contents (Cheikh-Rouhou, et al. 2008) and it is preferred by industries when oil hydrogenation is required.

Table 1: Fatty acids composition of Moringa oil:

Fatty acids	Name	Relative distribution
C 22	Behenic acid	6.36 %
C16	Palmitic acid	6.61 %
C18	Stearic acid	5.30 %
C:20:0	Arachidic acid	3.46 %
C 20:1 n-9	Gondoic acid	2.75 %
C 16:1 n-7	Palmitoleic acid	1.78 %
C 18:1 n-7	Vaccenic acid	5.86 %
C 18:1 n-9	Oleic acid	66.12 %
C 18:2 n-6	Linoleic acid	1.03 %

Chemical composition of Moringa acetone extract:

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in acetone extract of Moringa shown in Table (2). In the GC- MS analysis, ten bioactive phytochemical compounds were identified in the acetone extract of Moringa . The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. The fraction for Moringa acetone extract was characterized by large amounts of β -Hydroxyisovaleric acid and Chromone , 5-hydroxy-6,7,8-trimethoxy-2,3 dimethyl , which constituted 59.9% and 18.82% respectively ,

Table 2: Phytocomponents identified in the acetone extract of Moringa by GC-MS:

No	RT (min)	Compound	Area sum %
1	6.35	β -Hydroxyisovaleric acid	59.9
2	7.6	Psi-Cumene	3.44
3	7.726	Citroviol	3.72
4	8.132	Trans-2,3-dimethoxycinnamic acid	5.52
5	9.06	Carveol	3.66
6	14.15	Cis- α -bisabolene	0.71
7	14.75	Eremophilene	1.99
8	15.74	Chrysin	1.19
9	18.3	A-bisabolol	1.04
10	22.02	Chromone , 5-hydroxy-6,7,8-trimethoxy-2,3 dimethyl	18.82

Total phenolic, total flavonoids contents in seed oil and acetone extract:

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The phenolic contents of Moringa seed oil and acetone extract were found to be 79.33 and 62.67 (mg/g extract), respectively, while total flavonoids content were 24.9 and 4.21 (mg/g extract) respectively (Table 3). From our data we can reported that Moringa seed oil have high concentration of total phenolic compounds and total flavonoids content compared with the acetone extract of Moringa seed.

Table 3: Total phenolic and total flavonoids contents of Moringa seed oil and acetone extract:

Plant extract	Total phenolic content (mg/g extract)	Total flavonoids content (mg/g extract)
Moringa seed oil	79.33	24.9
Moringa acetone extract	62.67	4.21

Phenolic compounds are a class of antioxidant agents which act as free radical terminators and also involved in retardation of oxidative degradation of lipids (Pourmorad et al. 2006). In addition, (Odukoya et al. 2005) has reported a strong relationship between phenolic content and antioxidant activity in selected fruits and vegetables. Thus, the presence phenolic compounds in Moringa seed oil and Moringa acetone extract are an added value to its nutritional and health potential. Furthermore, the occurrence of flavonoids in the Moringa oil and Moringa acetone extract which are also phenolic compounds, similarly improves the economic and health potential of the seeds. This is in agreement with previous findings which suggested that flavonoids carry out antioxidant action through scavenging or chelating process and are reported to play a preventive role in cancer and heart disease (Middleton et al. 2000). Therefore, the importance of the antioxidant constituents of Moringa seeds in the maintenance of health is strengthened as trend of the future is moving toward using foods as medicine in the management of various chronic diseases.

Antioxidant activity of Moringa seed oil and acetone extract:

Reducing power activity:

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism in phenolic antioxidant action (Nabavi et. al. 2009). In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe^{+3} to Fe^{+2} by donating an electron. The amount of Fe^{+2} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 1 shows the dose– response columns for the reducing powers of the oil and acetone extract of Moringa seed. It was found that the reducing powers of oil and acetone extract also increased with an increase in their concentrations. At the highest concentration (100 μ g/ml) Moringa seed oil showed highest activity (0.666) while Moringa acetone extract was (0.267), at the same concentration. The observed increase in reducing power of the oil and acetone extract which were concentrations dependent suggested that they are good electron donors. Studies have shown that the reducing power capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Sofidiya et al. 2006); (Adesegun et al. 2008). This suggestion was confirmed in this present study whereby Moringa oil with more phenolic content exhibited greater antioxidant constituent and activity than Moringa acetone extract.

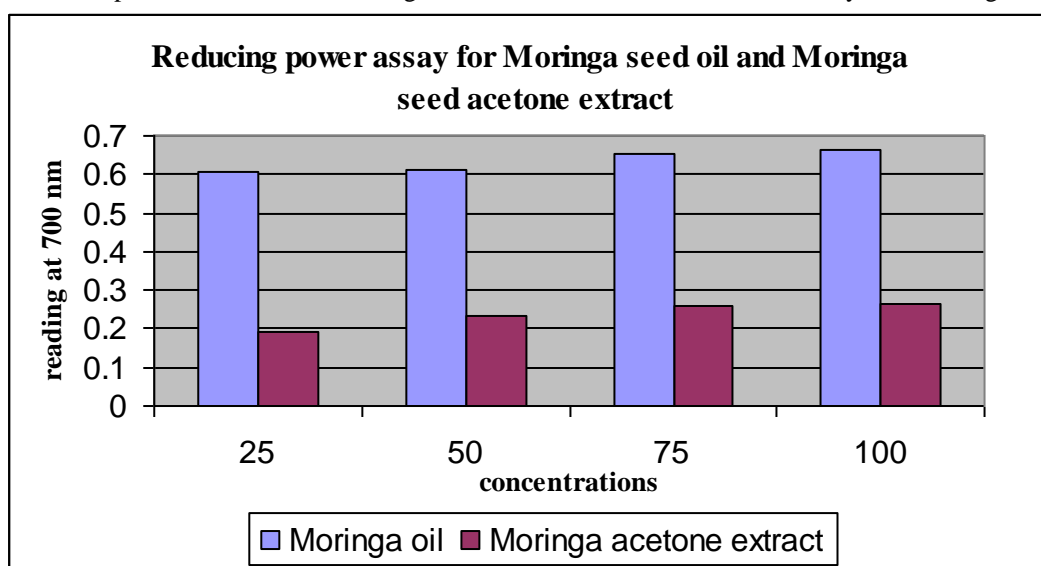


Fig. 1: Reducing power assay for Moringa seed oil and Moringa seed acetone extract:

2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen –or electron– donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour et. al. 2009). In fact, free radical scavenging method (DPPH) show the reduction of alcoholic DPPH solutions in the presence of an hydrogen donating antioxidant (Koleva et. al. 2002) and phenolic compound have been reported and provided to be potent hydrogen donors to the DPPH radical (Von et. al. 1997) because of their excellence structural chemistry (Rice-Evans et. al. 1997). The antioxidant potential of Moringa oil and Moringa acetone extract were further highlighted by the quenching of DPPH free radicals. The values of absorbance for seed oil and seed acetone extract ranged from 44.15 to 51.66 and 45.73 to 46.05 respectively compared with the values of absorbance for ascorbic acid ranged from 94.66 to 98.31 and followed the order of effectiveness as: ascorbic acid > Moringa seed oil > Moringa seed acetone extract . In general, the oil of the Moringa oil, exhibiting greater total phenolic and total flavonids content in the present analysis. The decrease in absorbance of DPPH-oil and acetone extract mixtures in this study which measured the extent of radical scavenging potential of the oil and extract supported the findings of (AsokKumar et al. 2009).

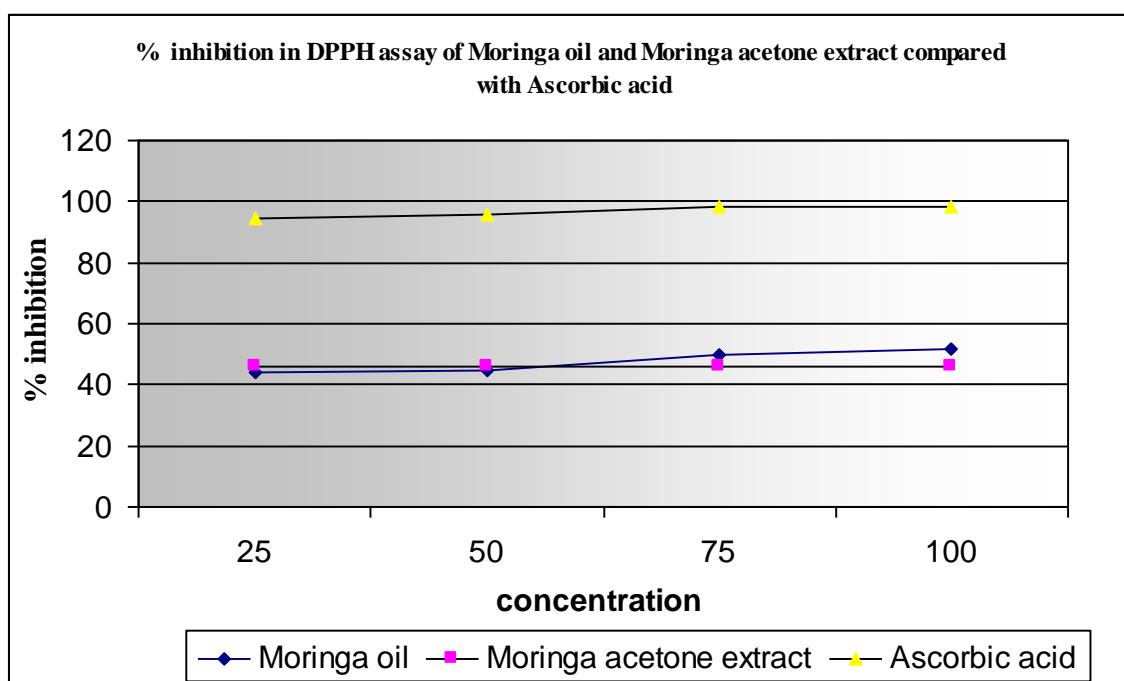


Fig 2: % inhibition in DPPH assay of Moringa oil and Moringa acetone extract compared with Ascorbic acid :

Protective effect of Moringa seed oil and Moringa seed acetone extract against ethanol administration:

Table (3) illustrate the effect of ethanol and/or supplemented Moringa seed oil and Moringa seed acetone extract on plasma liver functions parameters. In comparison with Negative zcontrol group in group treated with ethanol revealed significantly increased AST , ALT and ALP activities . In rats subjected to ethanol and supplemented with Moringa oil and seed acetone extract, the enzyme liver marker indicate a decrease of AST (94.40 ± 1.140 and 98.40 ± 1.140), ALT (23.80 ± 1.643 and 31.40 ± 3.362) and ALP (270.20 ± 1.304 and 304.00 ± 3.808), as compared with group treated with ethanol only.

Table 4: Effect of Moringa seed oil and Moringa seed acetone extract on AST , ALT and ALP activities in rats treated with ethanol for 30 days.

Treatment / parameter	ALT	AST	ALP
Negative control	25.00 ± 1.000 ^a	64.20 ± 1.095 ^a	268.20 ± 1.643 ^b
Positive control	36.80 ± 1.924 ^c	109.0 ± 2.646 ^e	329.60 ± 1.140 ^d
Moringa seed oil	23.80 ± 1.643 ^a	94.40 ± 1.140 ^c	270.20 ± 1.304 ^b
Moringa seed acetone extract	31.40 ± 3.362 ^b	98.40 ± 1.140 ^d	304.00 ± 3.808 ^c
Silymarin	25.60 ± 1.673 ^a	90.80 ± 1.304 ^b	269.40 ± 1.140 ^b

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.05$), while the same letters indicate non significant variation.

Data in Table (4) appeared a significant decrease ($P < 0.5$) in plasma albumin and total protein concentration in ethanol treated group comparing to control group with mean value of (3.840 ± .2702 , 6.000 ± .1581), (4.240 ± .2408 , 7.018 ± .2588) respectively. However, Moringa seed oil and Moringa seed acetone extract groups caused significant elevation ($P < 0.05$) in mean value of albumin parameter compared to ethanol group while total protein not affected by Moringa groups treatment . In the other hand bilirubin in ethanol treated group appeared significant elevation ($P < 0.05$) in mean value comparing to control group with mean value of (0.2080 ± 0.2168) , (0.1580 ± 0.3834) respectively. However, Moringa seed oil and Moringa seed acetone extract groups caused significant decrease ($P < 0.05$) in mean value of bilirubin parameter compared to ethanol group.

Table 5: Effect of Moringa seed oil and Moringa seed acetone extract on albumin , total protein and bilirubin in rats treated with ethanol for 30 days.

Treatment / parameter	Albumin	Total protein	bilirubin
Negative control	4.240 ± .2408 ^b	7.018 ± .2588 ^b	0.1580 ± .03834 ^a
Positive control	3.840 ± .2702 ^a	6.000 ± .1581 ^a	0.2080 ± .02168 ^b
Moringa seed oil	4.080 ± .1924 ^{ab}	6.420 ± .3271 ^a	0.1660 ± .02074 ^a
Moringa seed acetone extract	4.040 ± .2510 ^{ab}	6.040 ± .2302 ^a	0.1880 ± .02864 ^{ab}
Silymarin	4.200 ± .1871 ^b	6.440 ± .6914 ^a	0.1600 ± .02915 ^a

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.05$), while the same letters indicate non significant variation.

Ethanol is a chief ingredient in most of the syrups, tinctures, and other medicines. In small doses it is having a great medicinal value. But we know that most of the people in our society abuse ethanol (Zakhari and Li 2007). In excess doses, it causes severe hepatic damage in humans and experimental animals. Chronic administration of ethanol is known to have a profound effect on metabolism of lipids and lipoproteins. Moreover, this results in accumulation of hepatic lipids as well as lipid peroxides which lead to autooxidation of hepatic cells by disrupting the balance between the levels of pro-oxidants and antioxidants (Tsukamoto et. al. 2001) and (Zhou et. al. 2003). Therefore, this leads to oxidative stress in the hepatic cells which is the most striking initial manifestation of alcohol induced liver injury. When there is damage to the liver cell membrane, the cytosolic enzymes are leaked into the blood stream (Ramaiah 2007). Therefore, the elevation of these cytosolic enzymes in the blood stream is a needful quantitative marker of the extent of hepatic damage. This study investigated the protective role of Moringa oleifera seed oil against ethanol-induced hepatocellular oxidative damage. Treatment with the M. oleifera attenuated the elevated levels of AST, ALT, ALP , total bilirubin, total protein and albumin levels .Earlier studies demonstrated that root and flower of M. oleifera had reduced elevated AST, ALT and ALP levels in rodents (Al-Said et. al. 2012) and (Buraimoh et. al. 2011). And also another study has showed that leaf extracts of M. oleifera had significantly restored the elevated AST, ALT and ALP enzyme levels to the normal levels (Buraimoh et. al. 2011). Recently (Nadro et al. 2005) has demonstrated that M. oleifera leaves protect the hepatocytes by preventing the release of these 3 enzymes. Additionally, M. oleifera showed effective DPPH scavenging activity, suggesting that it could reduce the oxidative stress induced by chronic administration of ethanol. This finding is consistent with previous studies which demonstrated the antioxidant activity of M. oleifera extract (Santos et. al. 2012). The antioxidant property of M. oleifera may be due to bioactive phenolic compounds

in seeds .These compounds quench reactive oxygen species (ROS) and regenerate membrane-bound antioxidants levels during administration of *M. oleifera* at different dose levels in preventive study.

Effect of Moringa seed oil and Moringa seed acetone extract against ethanol on plasma antioxidants:

The concentration of malondialdehyde in the liver was taken as an index of hepatic lipid peroxidation induced by toxicant ethanol , while level of activity of catalase was determined as induce of hepatic antioxidant status. The results are presented in Table (5).

Table 6: Effect of Moringa seed oil and Moringa seed acetone extract on MDA and catalase activity in rats treated with ethanol for 30 days.

Treatment / parameter	MDA	Catalase
Negative control	10.16 a \pm 1.078	172.80 a \pm 1.304
Positive control	16.64 d \pm .434	256.40 e \pm 1.140
Moringa seed oil	15.28 c \pm .646	242.80 c \pm 1.304
Moringa seed acetone extract	15.52 c \pm .356	253.00 d \pm 2.915
silymarin	12.92 b \pm .898	193.80 b \pm 1.924

Each value is the mean \pm SD. Means have different superscript letters indicate significant variation at ($P \leq 0.05$), while the same letters indicate non significant variation.

It was found that ethanol intoxication caused a markedly elevated hepatic MDA level (Table 5) which is an indication of increased oxidative stress; this finding correlates with previous reports (Manna et. al. 2011). Previously, *Moringa oleifera* seed oil had been suggested to be capable of reversing or inhibiting lipid peroxidation in liver cells (Olatosin et. al. 2013). In the present study, animals pre administered with the oil and acetone extract of *Moringa* seeds had significantly lower MDA compared with those treated only with the toxicant ethanol, an indication of the attenuating effect of *Moringa oleifera* seeds on ethanol-induced liver injury. Catalase activity changes in the blood plasma implied that ethanol treatment increased CAT levels, and that *Moringa* seed oil and *Moringa* acetone extract tended to decrease CAT levels. Our findings are similar with (Lee and Ko 2012) who suggested that the plant extracts decreased CAT levels in rats through the same mechanism as that of the antioxidant ascorbic acid and that they have potential as antioxidants.

4- Conclusions:

The present study concludes that the seed oil and acetone extract of *M. oleifera* possesses antioxidant activity and shows a protective effect against ethanol induced hepatotoxicity in experimental rats. The antioxidant and hepatoprotective potential of *M. oleifera* seed oil and seed acetone extract may be attributed to the presence of total phenolics and flavonoids in the seeds.

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