Iosr Journal Of Pharmacy
E-Issn: 2250-3013, P-Issn: 2319-4219 Www.Iosrphr.Org
Volume 3, Issue 1 (February 2013), Pp 10-15

Evaluation Of *In Vitro* Antioxidant Activity and *In Vivo* Hepatoprotective Activity Of *Moringa Oleifera* Seeds Extract Against Ethanol Induced Liver Damage In Wistar Rats

[1]Eswar Kumar K[*], [1]Harsha K N, [1]Shabana Shaik, [2]Neelakanta Rao N, [1]Giri Babu N,
[1] *Pharmacology Division, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, Andhra Pradesh, India*
[2] *Rite Aid-store # 7198, 4212 Elvis Presley Blvd, Memphis, Tennesse 38116, USA*

Abstract: *The in vitro antioxidant and in vivo hepatoprotective effects of crude ethanolic extracts of Moringa oleifera (M. oleifera) seeds were evaluated in male Wistar rats against ethanol induced liver damage in preventive and curative models. The antioxidant activity of M. oleifera was assayed by DPPH, hydroxyl and superoxide radical scavenging activity. The various antioxidant activities were compared to standard antioxidant, ascorbic acid. In two different set of experiments, the M. oleifera extracts (50,100 and 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 25 days. In the 2,2-diphenyl-1-picrylhydrazil(DPPH), hydroxyl and superoxide radical scavenging activity, the IC_{50} values of ethanolic extract were 196.45 ± 0.25, 175.57 ± 0.39 and 213.15 ± 0.27 µg/ml respectively. The level of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were determined to assay hepatotoxicity. Ethanol administration caused severe hepatic damage in rats as evidenced by elevated serum AST, ALT, ALP and total bilirubin levels. The M. oleifera and silymarin administration prevented the toxic effect of ethanol on the above serum parameters in both preventive and curative models. The present study concludes that ethanolic extract of M. oleifera seeds has significant antioxidant and hepatoprotective activity against ethanol induced hepatotoxicity, which may be associated with its high bioactive compounds including glucosinolates, isothiocyanates, thiocarbamates, and flavonoids and antioxidant properties.*

Key words: *Antioxidant, Ethanol, Hepatoprotective, Moringa oleifera, Rat*

I. Introduction

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 proned to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotics[1]. 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 [2]. 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[3]. 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[4]. 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[5-7]. 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[8]. Lipid peroxidation by ethanol induces hepatic oxidative stress which has been identified to take part in a pathogenic role in Alcoholic Liver Disease (ALD)[9].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. Medicinally, herbal drugs have made a significant contribution for the treatment of hepatotoxicity[10-11]. 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[12]. In recent decades, the extracts of leaves, seeds and roots of *M. oleifera* have been extensively studied for many potential uses including hypotensive[13], anti-tumour[14],

hepatoprotective[15], analgesic activity[16] and antioxidant[17]. Keeping these folkloric claims and reports in view, the present study attempted to assess the possible hepatoprotective potential of the crude ethanolic seed extract of *M. oleifera* in ethanol-induced hepatotoxicity in rats.

II. Materials And Method:

a. Plant extract, Chemical and drugs

The crude ethanolic seed extract of *M. oleifera* was supplied by M/s. Laila Impex, Vijayawada, India. 2,2-diphenyl-1-picrylhydrazil (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Silymarin was obtained as a gift sample from Micro Labs, Bangalore, India. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin estimated kits were procured from Span Diagnostics, Surat, India. All other chemicals and reagents used were of analytical grade.

b. DPPH scavenging assay

The DPPH scavenging activity of *M. oleifera* was measured according to the method of Liu and Zhao[18]. The reaction mixture contained 2 ml of 95% ethanol, 0.1 M DPPH and 2 ml of the *M. oleifera* (50-300 µg/ml). The solution was incubated at 25°C for 15 min, and the absorbance of *M. oleifera* was determined at 517 nm. The antioxidant activity of *M. oleifera* extract was evaluated according to the following formula: Scavenging rate (%) = [1-A]/ A_0 X100, Where A was absorbance of *M. oleifera* extract and A_0 was the absorbance of DPPH solution.

c. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to the method of Winter bourn and Sutton[19]. The reaction mixture contained 1 ml of 0.15 M phosphate buffer saline (pH 7.4), 1 ml of 40 g/ml safranin, 1 ml of 0.945 mM EDTA-Fe (II), 1 ml of 3% (v/v) H_2O_2, and 0.5 ml of the *M. oleifera* (50-300 µg/ml). After incubating at 37°C for 30 min, the absorbance of the *M. oleifera* was measured at 560 nm. The IC_{50} value of *M. oleifera* is the effective concentration at which the hydroxyl radicals were scavenged by 50%. The hydroxyl radical- scavenging activity was expressed as: Scavenging rate (%) = [A_0-A_1]/ A_0 X 100, Where A_0 was absorbance of blank and A_1 was the absorbance of *M. oleifera* extract.

d. Superoxide radical scavenging assay

Superoxide anion radical scavenging activity was determined according to the method of Stewart and Bewley[20]. The reaction mixture (3 ml) contained 13 mM methionine, 10 mM riboflavin,75 M nitrobluetetrazolium, 100 mM EDTA, 50 mM phosphate buffer (pH 7.8), and the *M. oleifera* (50-300 µg/ml). After illuminating the reaction mixture with a fluorescent lamp at 25º C for 30 min, the absorbance of the *M. oleifera* was measured at 560 nm. The scavenging rate was calculated using the following formula: Scavenging rate (%) = [A_0-A]/ A_0 X100, where A was the absorbance of *M. oleifera* and A_0 was absorbance of the blank.

e. Animals

Adult male albino Wistar rats (180 ± 20 g) were obtained from the Mahaveer Enterprizes, Hyderabad, India. They were kept under temperature of (23 ± 2)ºC, humidity of 50% and 12 h:12 h of light and dark cycles, respectively. They were fed with Commercial pellet diet (Rayon's Biotechnology Pvt Ltd, India) and water was provided *ad libitum.* The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethics Committee and our lab was approved by CPCSEA, Government of India (Regd. No. 516/01/A/ CPCSEA).

f. *In vivo* Hepatoprotective Study

Preventive study:

The rats were divided into six groups each group containing 6 rats.

Group 1: Normal control rats which received 2% gum acacia for 25 days.

Group 2: Received 3.76 g/kg bw of ethanol for a period of 25 days.

Group 3: Received 3.76 g/kg bw of ethanol and 50 mg/kg bw of *M. oleifera* extract simultaneously for 25 days.

Group 4: Received 3.76 g/kg bw of ethanol and 100 mg/kg bw of *M. oleifera* extract simultaneously for 25 days.

Group 5: Received 3.76 g/kg bw of ethanol and 300 mg/kg bw of *M. oleifera* extract simultaneously for 25 days.

Group 6: Received 3.76 g/kg bw of ethanol and 100 mg/kg bw of silymarin simultaneously for 25 days.

Curative study:

Group 1: Normal control rats which received 2% gum acacia for 50 days.
Group 2: Received 3.76 g/kg bw of ethanol for a period of 50 days.
Group 3: Received 3.76 g/kg bw of ethanol daily for a period of 25 days and then received 50 mg/kg bw of *M. oleifera* extract for next 25 days.
Group 4: Received 3.76 g/kg bw of ethanol daily for a period of 25 days and then received 100 mg/kg bw of *M. oleifera* extract for next 25days.
Group 5: Received 3.76 g/kg bw of ethanol daily for a period of 25 days and then received 300 mg/kg bwof *M. oleifera* extract for next 25days.
Group 6: Received 3.76 g/kg bw of ethanol for 25 days and then silymarin 100mg/kg orally for next 25 days**.**
Administrations were done orally. Silimarin was the reference hepatoprotective agent. In preventive study, blood samples were collected on 0^{th} and 26^{th} day and in curative study, blood samples were collected on 0^{th}, 26^{th} and 51^{st} day from rats retro-orbital plexus. Blood samples were collected into centrifuge tubes and were centrifuged at 3000 rpm for 30 min to obtain the serum, used for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin in Semi-auto analyzer (Screen master-3000).

III. Results

g. Effect of *M. oleifera* against the DPPH radicals

The various concentrations of *M. oleifera* and standard ascorbic acid in the dose range of 50- 300 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *M. oleifera* and ascorbic acid were found to be 196.45 µg/ml and 148.95 µg/ml respectively.

h. Effect of *M. oleifera* against the hydroxyl radicals

The various concentrations of *M. oleifera* and standard ascorbic acid in the dose range of 50- 300 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *M. oleifera* and ascorbic acid were found to be 175.57 µg/ml and 143.95 µg/ml respectively.

i. Effect of *M. oleifera* on the superoxide scavenging activity

The various concentrations of *M. oleifera* and standard ascorbic acid in the dose range of 50- 300 µg/ml showed antioxidant activity in a dose dependent manner. The IC_{50} values for *M. oleifera* and ascorbic acid were found to be 213.15 µg/ml and 139.08 µg/ml respectively.

j. Estimation of serum biochemical parameters

Results presented in Table 1 to 4 indicate that the levels of serum enzymes namely AST, ALT, ALP and total bilirubin levels were significantly ($p<0.01$) increased in ethanol treated rats when compared with normal rats. However, treatments of rats with *M. oleifera* and silymarin serum enzymes like AST, ALT, ALP and total bilirubin levels were significantly ($p<0.01$) decreased when compared to ethanol treated rats in both preventive and curative study.

IV. Discussion

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[4]. 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[6-7]. 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[21]. Therefore, the elevation of these cytosolic enzymes in the blood stream is a needful quantitative marker of the extent of hepatic damage. The elevated levels of the ASP, ALT, ALP and total bilirubin levels in the rats administered with ethanol indicate the hepatocellular damage and alterations in the membrane permeability. Our reports on these elevated levels during ethanol induced hepatic damage are in accordance with the previous reports[22]. Pre-treatment and after-treatment with the *M. oliefera* attenuated the elevated levels of ASP, ALT, ALP and total bilirubin levels.Earlier studies demonstrated that root and flower of *M. oleifera* had reduced elevated AST, ALT and ALP levels in rodents[15, 23]. 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[23]. Recently Nadro et al.[24] has demonstrated that *M. oleifera* leaves protect the hepatocytes by preventing the release of these 3 enzymes. Our results are consistent with earlier studies, which strongly suggest that *M. oleifera* may protect the structural integrity of hepatocytes and prevent the release of cytosolic enzymes into bloodstream.Additionally, *M. oleifera* showed effective DPPH, hydroxyl, super oxide radical 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[17]. The antioxidant property of *M. oleifera* may be due to bioactive compounds glucosinolates, isothiocyanates, thiocarbamates, and flavonoids in seeds[26].These compounds quench ROS and regenerate membrane-bound antioxidants levels during administration of *M. oleifera* at different dose levels in preventive and curative studies.

V. Conclusion

The present study concludes that the ethanolic seed extract of *M. oleifera* possesses antioxidant activity and shows a protective effect against ethanol induced hepatotoxicity in experimental rats. However, further investigation is in process on the seeds extract to identify the active constituents' responsibility for hepatoprotection.

VI. Acknowledgements

The authors thank M/s. Lailaimpex, Vijayawada, India for providing gift sample of ethanolic seed extract of *Moringa oleifera* plant extract.

References

[1] Amacher, DE. A Toxicologist's Guide To Biomarkers Of Hepatic Response. Hum Exp Toxicol, 21(5), 2002; 253-262.
[2] Salhab, M And Canelo, R. An Overview Of Evidence-Based Management Of Hepatocellular Carcinoma: A Meta-Analysis. J Cancer Res Ther, 7(4), 2011; 463-475.
[3] Mcdonough, KH. Antioxidant Nutrients And Alcohol. Toxicology, 189, 2003; 89-97.
[4] Zakhari, S And Li, TK. Determinants Of Alcohol Use And Abuse: Impact Of Quantity And Frequency Patterns On Liver Disease. Hepatology, 46, 2007; 2032-2039.
[5] Thurman, RG. Alcoholic Liver Injury Involves Activation Of Kupffer Cells By Endotoxin. American Journal Of Physiology, 275, 1998; 605-611.
[6] Tsukamoto, H. Takei, Y. Mcclain, CJ. Shimizu, H. Sato, N And Thurman R. How Is The Liver Primed Or Sensitized For Alcoholic Liver Disease. Alcohol Clinical Express Research, 25, 2001; 171-181.
[7] Zhou, Z. Wang, L. Song, Z. Lambert, JC. Mcclain, CJ And Kang YJ. A Critical Involvement Of Oxidative Stress In Acute Alcohol-Induced Hepatic TNF-Alpha Production. American Journal Of Pathology, 163, 2003; 1137-1146.
[8] Crawford, DW And Blankenhorn, DH. Arterial Wall Oxygenation, Oxyradicals And Atherosclerosis. Atherosclerosis, 89, 1991; 97-108.
[9] Bunout, D. Nutritional And Metabolic Effects Of Alcoholism: Their Relationship With Alcoholic Liver Disease. Nutrition, 15, 1999; 583-589.
[10] Li, R. Guo, W. Fu, Z. Ding, G. Zou, Y And Wang Z. Hepatoprotective Action Of Radix Paeoniae Rubra Aqueous Extract Against Ccl4-Induced Hepatic Damage. Molecules, 16(10), 2011; 8684-8694.
[11] Yen, FL. Wu, TH. Lin, LT And Lin, CC. Hepatoprotective And Antioxidant Effects Of Cuscuta Chinensis Against Acetaminophen-Induced Hepatotoxicity In Rats. J. Ethnopharmacol, 111, 2007; 123-128.
[12] L.J. Fuglie, The Miracle Tree. Moringa Oleifera: Natural Nutrition For The Tropics, Church World Service, Dakar, Senegal,1999, 63.
[13] Faizi, S. Siddiqui, BS. Saleem, R. Siddiqui, S. Aftab, K And Gilani, AH. Fully Acetylated Carbamate And Hypotensive Thiocarbamate Glycosides From Moringa Oleifera.Phytochemistry, 38(4),1995; 957-963.
[14] Guevara, AP. Vargas, C, Sakurai, H, Fujiwara, Y, Hashimoto, K And Maoka, T, Et Al. An Antitumor Promoter From Moringa Oleiferalam. Mutatant Research, 440, 1999; 181-188.
[15] Al-Said, MS. Mothana, RA. Al-Yahya, MA. Al-Blowi, AS. Al-Sohaibani, M. Ahmed, AF And Rafatullah, S. Edible Oils For Liver Protection: Hepatoprotective Potentiality Of Moringa Oleifera Seed Oil Against Chemical-Induced Hepatitis In Rats. J Food Sci, 77(7), 2012; T124-130.
[16] CV. Rao, And SK. Ojha, Analgeslc Effect Of Moringa Oleifera Lam Leaf Extract On Rats. 2nd World Congress On Biotechnological Developments Of Herbal Medicine Lucknow. India. NBRI 2003. P 42.MAPA - 02-911.
[17] Santos, AF. Argolo, AC. Paiva, PM And Coelho LC. Antioxidant Activity Of Moringa Oleifera Tissue Extracts. Phytother Res, 26(9), 2012; 1366-1370.
[18] Liu, X And Zhao, M. Antioxidant Activities And Functional Composition Content Of Selected Phyllanthusemblica Fruits Juice. Food Fermentation Industries, 5: 2006; 151-154.
[19] Winterbourn, CC And Sutton, HC. Hydroxyl Radical Production From Hydrogen Peroxide And Enzymatically Generated Paraquat Radicals: Catalytic Requirements And Oxygen Dependence. Arch. Biochem. Biophys, 235, 1984; 116-126.
[20] Stewart, RC And Bewley, JD. Lipid Peroxidation Associated With Accelerated Aging Of Soybean Axes. Plant Physiol, 65(2), 1980; 245-248.
[21] Ramaiah, SK. A Toxicologist Guide To The Diagnostic Interpretation Of Hepatic Biochemical Parameters. Food Chem. Toxicol, 45 (4), 2007; 1551-1557.
[22] Sridevi, VK. Chouhan, HS. Singh, NK And Singh SK. Antioxidant And Hepatoprotective Effects Of Ethanol Extract Of Vitex Glabrata On Carbon Tetrachloride-Induced Liver Damage In Rats. Nat Prod Res, 26(12), 2012; 1135-1140.
[23] Buraimoh, AA. Bako, IG And Ibrahim, FB. Hepatoprotective Effect Of Ethanolic Leave Extract Of Moringa Oleifera On The Histology Of Paracetamol Induced Liver Damage In Wistar Rat. International Journal Of Animal And Veterinary Advances, 3(1), 2011; 10 -13.
[24] Nadro, MS. Arungbemi, RM And Dahiru, D. Evaluation Of Moringa Oleifera Leaf Extract On Alcohol-Induced Hepatotoxicity. Trop. J. Pharmaceut. Res, 5(1), 2005; 539-544.

Table 1: Effect of M. *oleifera* ethanolic seeds extract and silymarin on AST and ALT on ethanol induced hepatotoxicity in wistar rats (preventive study)

Groups	AST		ALT	
	0 day	26th day	0 day	26th day
Normal control rats	$36.5^{ns} \pm 3.6$	$37.4^{ns} \pm 3.1$	$32.6^{ns} \pm 3.2$	$33.6^{ns} \pm 4.2$
ETH control (3.76 g/kg bw)	$40.6^{ns} \pm 4.5$	$195.5^{\#} \pm 5.6$	$36.4^{ns} \pm 3.6$	$150.2^{\#} \pm 5.7$
ETH (3.76 g/kg bw)+ M.O (50 mg/kg bw)	$48.5^{ns} \pm 2.1$	$143.4^{*} \pm 2.5$	$32.6^{ns} \pm 3.2$	$82.8^{*} \pm 2.9$
ETH (3.76 g/kg bw)+ M.O (100 mg/kg bw)	$46.8^{ns} \pm 4.3$	$119.4^{*} \pm 4.6$	$28.5^{ns} \pm 2.5$	$62.2^{*} \pm 2.2$
ETH (3.76 g/kg bw)+ M.O (300 mg/kg bw)	$43.6^{ns} \pm 3.5$	$92.7^{*} \pm 3.1$	$30.1^{ns} \pm 5.2$	$49.8^{*} \pm 3.7$
ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)	$32.7^{ns} \pm 3.2$	$45.6^{*} \pm 2.5$	$33.6^{ns} \pm 4.1$	$53.6^{*} \pm 4.2$

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; ETH= Ethanol; M.O= *M. oleifera*
#P<0.01, compared with normal control
*P<0.01, compared with ETH control

Table 2: Effect of M. *oleifera* ethanolic seeds extract and silymarin on ALP and total bilirubin on ethanol induced hepatotoxicity in wistar rats (preventive study)

Groups	ALP		Total bilirubin	
	0 day	26th day	0 day	26th day
Normal control rats	$46.8^{ns} \pm 4.2$	$48.3^{ns} \pm 3.8$	$0.41^{ns} \pm 0.01$	$0.42^{ns} \pm 0.02$
ETH control (3.76 g/kg bw)	$43.2^{ns} \pm 4.8$	$229.6^{\#} \pm 5.4$	$0.52^{ns} \pm 0.03$	$3.12^{\#} \pm 0.06$
ETH (3.76 g/kg bw)+ M.O (50 mg/kg bw)	$48.7^{ns} \pm 5.1$	$217.8^{*} \pm 6.2$	$0.51^{ns} \pm 0.06$	$1.13^{*} \pm 0.03$
ETH (3.76 g/kg bw)+ M.O (100 mg/kg bw)	$50.8^{ns} \pm 2.9$	$191.8^{*} \pm 2.8$	$0.58^{ns} \pm 0.03$	$0.94^{*} \pm 0.01$
ETH (3.76 g/kg bw)+ M.O (300 mg/kg bw)	$52.4^{ns} \pm 4.2$	$162.8^{*} \pm 2.5$	$0.54^{ns} \pm 0.04$	$0.78^{*} \pm 0.02$
ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)	$48.5^{ns} \pm 3.5$	$96.7^{*} \pm 3.4$	$0.43^{ns} \pm 0.01$	$0.75^{*} \pm 0.01$

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; ETH = Ethanol; M.O = *M. oleifera*
#P<0.01, compared with normal control
*P<0.01, compared with ETH control

Table 3: Effect of M. *oleifera* ethanolic seeds extract and silymarin on AST and ALT on ethanol induced hepatotoxicity in wistar rats (curative study)

Groups	AST			ALT		
	0 day	26th day	51st day	0 day	26th day	51st day
Normal control rats	$32.4^{ns} \pm 3.6$	$33.5^{ns} \pm 3.2$	$34.6^{ns} \pm 3.5$	$34.8^{ns} \pm 2.9$	$39.4^{ns} \pm 4.4$	$40.3^{ns} \pm 3.2$
ETH control (3.76 g/kg bw)	$32.6^{ns} \pm 3.1$	$205.8^{\#} \pm 2.8$	$301.2^{\#} \pm 2.1$	$30.5^{ns} \pm 3.9$	$162.5^{\#} \pm 8.4$	$365.4^{\#} \pm 9.3$
ETH (3.76 g/kg bw)+ M.O (50 mg/kg bw)	$40.5^{ns} \pm 2.2$	$188.6^{*} \pm 2.5$	$198.4^{*} \pm 1.8$	$32.8^{ns} \pm 2.6$	$126.2^{*} \pm 3.9$	$271.6^{*} \pm 3.9$
ETH (3.76 g/kg bw)+ M.O (100 mg/kg bw)	$42.6^{ns} \pm 4.2$	$157.4^{*} \pm 6.4$	$114.7^{*} \pm 2.7$	$33.5^{ns} \pm 3.5$	$107.8^{*} \pm 2.4$	$258.3^{*} \pm 2.6$
ETH (3.76 g/kg bw)+ M.O (300 mg/kg bw)	$43.7^{ns} \pm 5.2$	$106.6^{*} \pm 5.3$	$98.6^{*} \pm 3.8$	$26.7^{ns} \pm 2.6$	$95.3^{*} \pm 2.7$	$242.7^{*} \pm 2.4$
ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)	$44.3^{ns} \pm 5.8$	$95.7^{*} \pm 5.9$	$45.3^{*} \pm 2.3$	$28.5^{ns} \pm 1.5$	$63.5^{*} \pm 7.4$	$140.8^{*} \pm 1.6$

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; ETH = Ethanol; M.O = *M. oleifera*
#P<0.01, compared with normal control
*P<0.01, compared with ETH control

Table 4: Effect of M. *oleifera* ethanolic seeds extract and silymarin on ALP and total bilirubin on ethanol induced hepatotoxicity in wistar rats (curative study)

Groups	ALP			Total bilirubin		
	0 day	26th day	51st day	0 day	26th day	51st day
Normal control rats	$56.8^{ns} \pm 5.6$	$58.3^{ns} \pm 2.2$	$59.7^{ns} \pm 3.4$	$0.5^{ns} \pm 0.01$	$0.52^{ns} \pm 0.05$	$0.56^{ns} \pm 0.5$
ETH control (3.76 g/kg bw)	$52.2^{ns} \pm 4.9$	$240.7^{\#} \pm 11.7$	$460.3^{\#} \pm 7.2$	$0.6^{ns} \pm 0.04$	$3.92^{\#} \pm 0.31$	$4.84^{\#} \pm 0.3$
ETH (3.76 g/kg bw)+ M.O (50 mg/kg bw)	$58.6^{ns} \pm 2.4$	$214.3^{*} \pm 9.4$	$310.8^{*} \pm 6.8$	$0.63^{ns} \pm 0.03$	$1.54^{*} \pm 0.03$	$3.75^{*} \pm 0.2$
ETH (3.76 g/kg bw)+ M.O (100 mg/kg bw)	$53.7^{ns} \pm 4.8$	$172.7^{*} \pm 7.6$	$242.6^{*} \pm 7.3$	$0.41^{ns} \pm 0.01$	$0.86^{*} \pm 0.08$	$3.52^{*} \pm 0.3$
ETH (3.76 g/kg bw)+ M.O (300 mg/kg bw)	$62.8^{ns} \pm 3.1$	$144.6^{*} \pm 3.7$	$222.8^{*} \pm 2.5$	$0.48^{ns} \pm 0.02$	$0.62^{*} \pm 0.01$	$3.32^{*} \pm 0.1$
ETH (3.76 g/kg bw) + silymarin (100 mg/kg bw)	$58.5^{ns} \pm 4.8$	$112.9^{*} \pm 6.2$	$152.7^{*} \pm 2.1$	$0.6^{ns} \pm 0.01$	$0.53^{*} \pm 0.05$	$3.11^{*} \pm 0.3$

Values are expressed as Mean ± SD of 6 individuals.
ns= non significant; ETH = Ethanol; M.O = *M. oleifera*
#P<0.01, compared with normal control
*P<0.01, compared with ETH control