Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

Hepatoprotective Effect of Methanolic Extract of Origanum Majorana on Paracetamol-Induced Liver Toxicity in Rats

Manish Srivastava*[1], Dr. Lavkesh Kumar Omray, Dr. Naveen Gupta[1], Dr. Dharmendra S Rajput[1], Dr. Kapil Malviya[1]

[1]Patel college of pharmacy, Madhyanchal professional university, Bhopal, M.P.

Corresponding Author: Manish Srivastava

Patel college of pharmacy, Madhyanchal Professional University, Bhopal, M.P.

(Received: 08 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)

KEYWORDS

Origanum majorana, paracetamol-induced liver injury, Hepatoprotective effect, hesperetin

ABSTRACT:

Origanum majorana is a medicinal plant with traditional claims but ignored investigation regarding its hepatoprotective effects. The current study is aimed to investigate the hepatoprotective potential of Origanum majorana methanol, water extract and isolated compound (hesperetin) on paracetamol-induced liver injury. The phytochemical screening was done which showed the presence of potential constituents including flavonoids and phenols. For investigating the hepatoprotective effect of Origanum majorana extract, rats were given different treatments for seven consecutive days. The normal control was administered with normal saline, group 2 received paracetamol and group 3 (Standard) was given silymarin as reference drug. In group 4 and 5 (Treated), methanolic extract (100 and 200mg/kg); group 6 and 7 (Treated) aqueous extract (100 and 200mg/kg); group 8 (hesperetin) were administered. Different serum biomarkers and histopathological studies were performed to assess the recovery caused by paracetamol in comparison to diseased group. The treatment of Origanum majorana methanolic extract and (hesperetin) significantly improve the serum biomarkers and restored the hepatic injury towards normal, indicating the hepatoprotective potential. Thus, we can conclude that Origanum majorana and isolated compound (hesperetin) have significantly reversed the damage caused by paracetamol in hepatotoxic rat model

Introduction:

Liver disease can be caused by alcohol, drugs, environmental toxins, or viruses. The basic organ responsible for the biosynthesis, levels, and degradation of circulating biochemical compounds (proteins, enzymes, and hormones) is the liver. Consequently, the liver should be targeted in order to alter and alleviate the risk of different chronic illnesses [1, 2]. Liver injury continues to be among the most common of internal diseases in clinical settings. Hepatoprotectors occur a key place in the treatment of diseases, as well as in the treatment of virtually all other liver diseases. Hepatoprotectors are complex drugs of mainly plant origin intended to increase the resistance of the liver to toxic effects; they help to restore its functions and normalize or enhance the activity of liver cell enzymes [3, 4].

The potential use of higher plants as a source of new drugs is still poorly explored. In most cases, only pharmacological screening or preliminary studies have been carried out and out of estimated only 5000 species have been studied for their medicinal use. The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature. Advances in the methodologies used to evaluate extracts and pure compounds for biological activity has enabled the possibility to isolate and identify interesting biologically active compounds on sub milligram scale [5, 6].

Origanum majorana, also known as Marjoram, Sweet Marjoram or Knotted Marjoram, is a semi-evergreen, aromatic, small herbaceous perennial that forms a compact mound. Before blooming, the clusters of flower buds look like knots, hence the name “knotted marjoram. It has dense foliage of grey-green leaves and

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

erect flower stems bearing clusters of tiny white to pale pink flowers. The leaves are small and very smooth due to the numerous hairs. The colour is grey-green and becomes silvery grey during the summer. They are entire, elliptical and generally obtuse, with marked veins on the underside and almost parallel to the midrib. The petiole is short and hairy [7,8].

The fragrance is more intense when the leaves are rubbed or crushed. The leaf is, on average, 15 to 18 mm long and 3 to 6 mm wide. The flowers are grouped in spiked or rounded clusters arranged in a loose panicle along the stem. The flowers are tiny and usually white or pale pink. They are two-lipped, upper lip is notched, and the lower lip has 3 lobes. The corolla is 4 to 5 mm and is inserted between tightly packed grey-green bracts. The flowers bloom during the Summer. The stems are upright or spreading, green to reddish-green, hairy, and woody at the base. They are squared, with opposite grey-green leaves at each node. Clusters of tiny white flowers with grey-green bracts are arranged in panicles with multiple branched stems growing from a central stem. In present study, we investigated the protective effects of a Origanum majorana, extract of paracetamol-induced liver damage [9-11].

MATERIAL AND METHODS

Extraction of plant material: The crude plant material was often subjected to the selective extraction comprised of treating the moderately coarse powder of plant material with non polar solvent in succession to extract various plant constituents according to their solubility. The powdered plant material (250 gm) were successively extracted in a soxhlet apparatus with petroleum ether (60-80°C), methanol and finally with water (by maceration process).

Isolation and characterization of compounds from Origanum majorana leaves extract

The methanol extract was chromatographed on silica gel and eluted with hexane, followed by increasing concentrations of ethyl acetate and methanol in hexane(0–100% ethyl acetate and methanol), respectively, to collected 50 fractions. The fractions were checked by TLC to determine retention values (Rf) of the compounds. A particular compound will travel the same distance along the stationary phase by a specific solvent. Therefore the fractions containing compounds having the same Rf value were combined. Finally seven fractions were obtained. After mixing the fractions to obtain the same pure compounds, some impurities were detected. Subfraction four were showed one major spot which were further purified by preparative TLC.

Hesperetin (compound II) was obtained as white needles. Its molecular formula was based on C16H14O6 by MS (m/z 301.1042 [M−H]). UV spectroscopy indicated the presence of a flavonone at 288 and 330 nm. IR absorption suggested the presence of a hydroxy group (3475 cm−1) and a carbonyl group (1641 cm−1). The 1H-NMR spectrum showed signals at δ 2.71 (1H, dd, J =3.1 Hz, J =17.1 Hz), indicating the existence of single bond and H3 proton coupled with H3′ and H2. The signals observed at δ 5.43 (dd, J =3.1 Hz, J =17.1 Hz) belonged to H2, which coupled with H3 and H3′. H6 resonated at δ 6.08 (1H, d, J =2.2 Hz), which coupled with H8 as meta coupling. The signals appeared at δ 6.10 as a doublet which coupled with H6 belonged to H8. Based on these data, the compound can be assigned as hesperetin

Hepatoprotective activity

Animal Housing and environmental condition: Animals wistar rats weighing between 180-200 gm were selected for in vivo pharmacological screening of Origanum majorana leaves extracts. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC Approval No.: IIP/CPCSEA/IEAC/2023/02) and ethical norms were strictly followed during all experimental procedures

Dosing protocol

For investigating the hepatoprotective effect rats were given different treatments for seven consecutive days. The normal control was administered with normal saline, group 2 received paracetamol and group 3 (Standard) was given silymarin as reference drug. In group 4 and 5 (Treated), methanolic extract (100 and 200mg/kg); group 6 and 7 (Treated) aqueous extract (100 and 200mg/kg); group 8 (hesperetin 10mg/kg) were administered.

Normal control: Normal saline

Disease control: Paracetamol 640 mg/kg p.o. for 7 days

Standard: Standard (silymarin treated)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

MEOM 100: Methanol extract of Origanum majorana leaves (100 mg/kg)

MEOM 200: Methanol extract of Origanum majorana leaves (200 mg/kg)

AQOM 100: Aqueous extract of Origanum majorana leaves (100 mg/kg)

AQOM 200: Aqueous extract of Origanum majorana leaves (200 mg/kg)

Hesperetin: Hesperetin (10 mg/kg)

Blood and liver samples

Twenty-nine days after the start of the experiment, the rat were anesthetized with nembutal (50 mg/kg, i.p.) and sacrificed. Fresh blood was collected directly from the heart in cold EDTA containers (5 cm3Monovette, Germany). After centrifugation of blood samples (4,000 rpm at 4°C for 10 min), 200 µL of plasma from each group was stored at – 40°C until further assay. The freshly collected liver (unextravasation with cold 0.9% saline) of all animals in each group was stored on ice. After homogenization and addition of solvents, samples were centrifuged at 4,000 rpm at 4°C for 10 min, and 300 µL of the supernatant was stored at – 4°C until further assay

Mortality: All the animals were observed twice daily (morning and evening) for morbidity and mortality, throughout the acclimatization and period of study.

Clinical Signs: After test item administration, individual animals were observed for abnormal clinical signs, due to treatment, throughout the study period. The clinical observations included changes in skin and fur, in the eyes and mucosal membrane in the respiratory, circulatory, central nervous and autonomous system and behaviour.

Body weight: Body weight was recorded on day 0 (prior to dosing) and day 8. Change in body weight (%) was calculated on day 8 based on previous body weight

Biochemical Analysis: Using rat capillaries, blood was drawn from retro-orbital plexus and was transferred to the in heparinised eppendorf tubes. It was then centrifuged at 3000 RPM for 10 minutes to separate plasma, which was collected using clean pipette. After collection, plasma samples were subjected to biochemical analysis. Biochemical analysis was performed on blood of animals fasted overnight. The major reason for this is the increased variability that would inevitably result from feeding and would mask more subtle effects and make interpretation difficult.

The biochemical parameters from the samples of blood plasma were evaluated using automated analyzer (ERBA Diagnostc Mannheim GmbH; Model: CHEM-7). The parameters Amino transaminases, which included, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) were analyzed. These enzymes are found mainly in liver, but are also found in red blood cells, heart cells, muscle tissue blood plasma and other organs like pancreas and kidney. Elevated levels of AST and ALT indicated disease or injury to the liver.

Oxidative stress parameters: The liver was perfused with 0.86% cold saline to completely remove all the blood cells. The liver was blotted, washed and divided into parts to be used for different analyses. For this, 0.1g of the liver was homogenized in 0.1m ice-cold phosphate buffer (pH 7.4), centrifuged and the supernatant was collected. The following oxidative stress parameters were evaluated from tissue homogenate of liver.

Level of lipid peroxidation: Malondialdehyde (MDA) is a marker of oxidative stress and is often measured as a marker of oxidative stress in peripheral veins as a reflection of hepatic oxidative stress. Activity of superoxide dismutase (SOD) is an important antioxidant enzyme, which rapidly catalyzes the dismutation of superoxide anion and thus acts as a first line antioxidant defense. Lower SOD activity indicates oxidative stress.

E. Liver weight: Liver weight of all group animals was recorded after scarification. liver weight of all groups animal were calculated. The absolute weight of liver of each rat was recorded after the terminal sacrifice. Liver tissues of all animals were collected in normal saline before weighing. Fat was trimmed and the weighing of wet tissue was done using Electronic weighing balance (Sartorius Cubis analytical balance).

Results and discussion

Clinical signs and mortality: Administration of paracetamol, Origanum majorana extracts and isolated compounds showed no mortality or morbidity in the animals during the period of study. Cage side

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

observations did not show any observable clinical signs related to the compound toxicity. No tremors, convulsions, salivation, diarrhoea, lethargy, or unusual behaviors were observed in extract treated animals throughout the study period.

Effect on body weight: The change in the body weights of the experimental animals from day 0 to day 8. Treatment with paracetamol induce hepatotoxicity caused significant reduction ($P<0.01$) in body weight

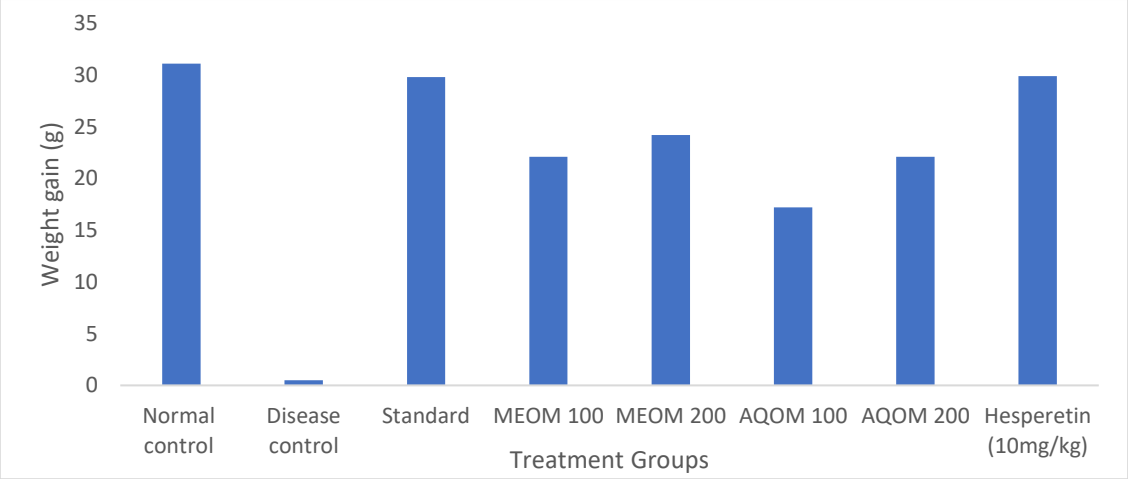
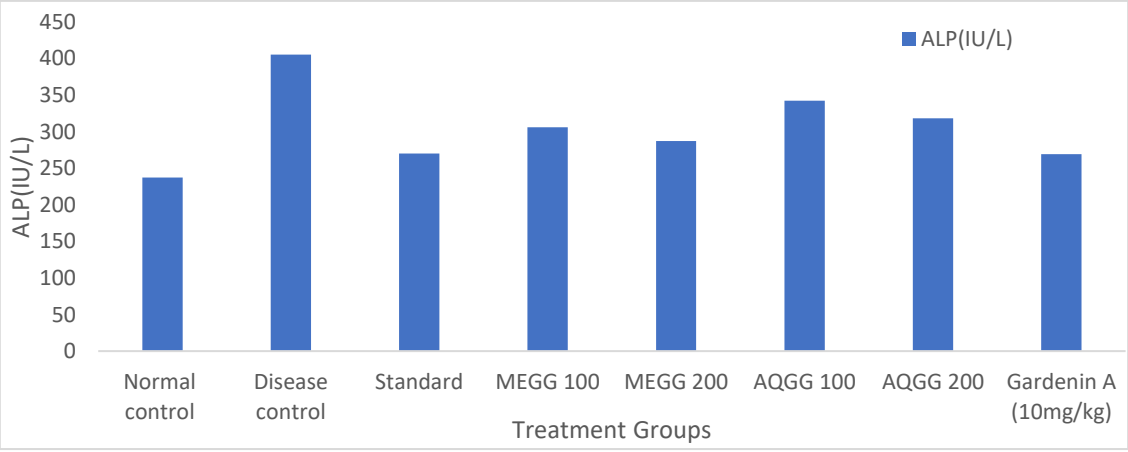


Figure 1: Effect on body weight

Effect of plants extract on the activity levels of AST and ALT in blood plasma:

Alkaline phospatase (ALP) enzyme is mainly from the liver and is present in its mucosal epithelium. Hepatic disease or injury may cause rise in the levels of ALP in blood plasma. Bilirubin, is the catabolic product of haemoglobin, released in unconjugaed form, from the liver. In case of hepatocellular damagetoxicity or injury, higher levels of bilirubin is observed in the blood plasma.

The levels of AST activity in the blood plasma of animals in different experimental groups were performed. Treatment with Origanum majorana leaves extracts control AST level in dose dependent manner. Origanum majorana leaves methanol extract control the AST level more efficiently compared to aqueous extract treated group. Treatment with isolated compound (Hesperetin) control the AST level similar to standard treated group.

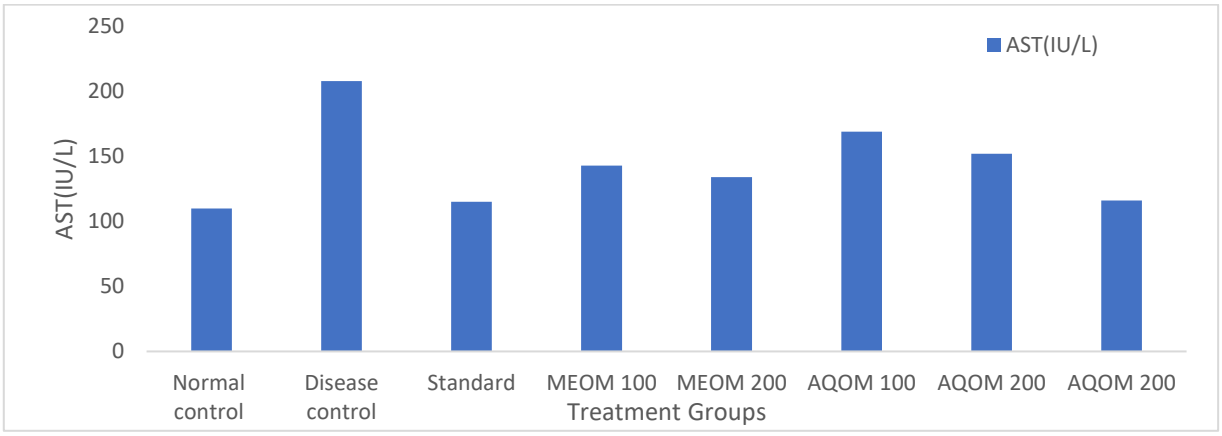


Effect of Origanum majorana leaves extract on ALP levels in blood plasma of animals

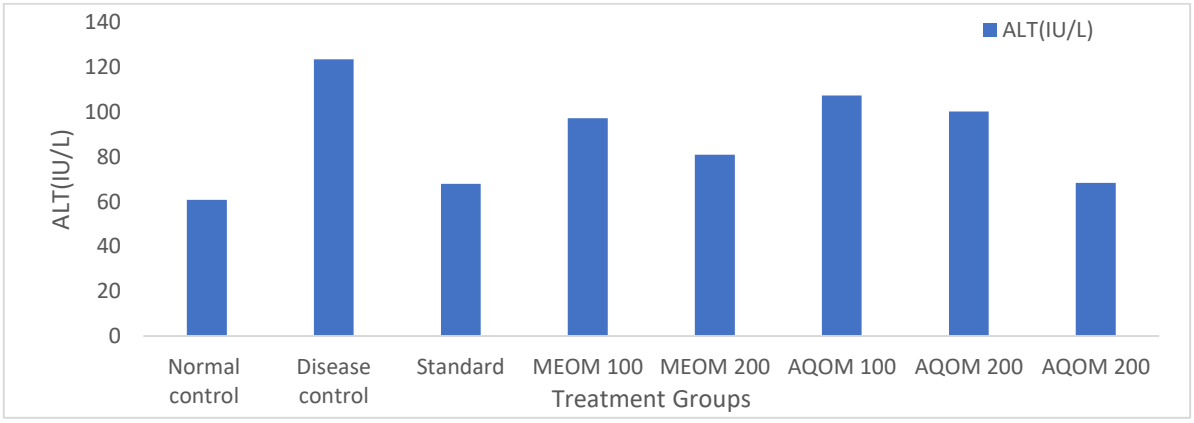
Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727



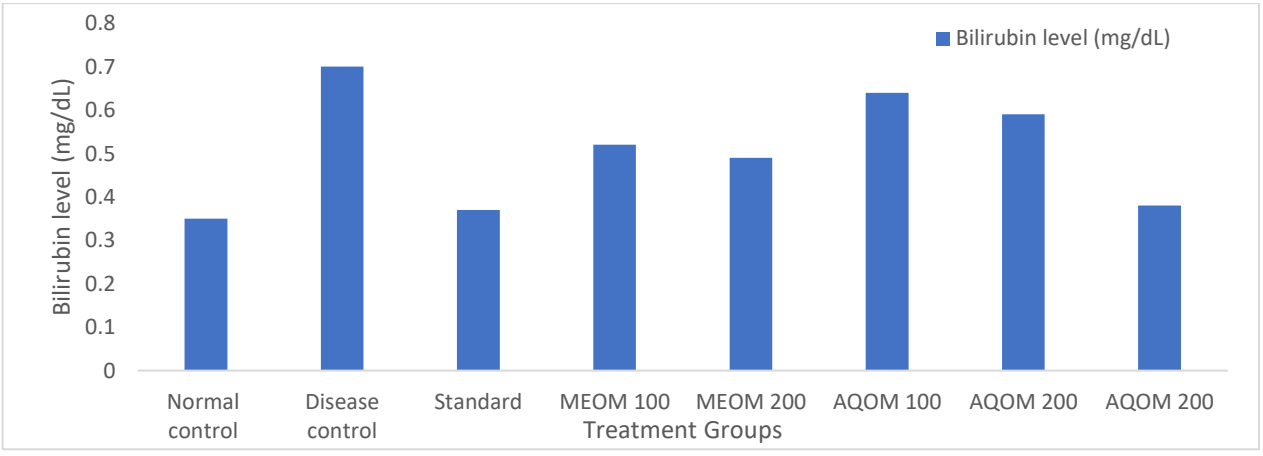
Effect of Origanum majorana leaves extract on AST levels in blood plasma of animals



Effect of Origanum majorana leaves extract on ALT levels in blood plasma of animals

Effect on Bilirubin level

The methanol extracts of Origanum majorana leaves extracts showed reduced Bilirubin level in blood plasma of animals. Administration of isolated compound (Hesperetin) control the Bilirubin level similar to standard treated group. This too suggests that the methanol extract of Origanum majorana leaves at the dose of 200 mg/kg and isolated compounds are also able to relieve the symptoms of hepatotoxicity.



Effect of Origanum majorana leaves extract on Bilirubin level in blood plasma of animals

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

Effect of extract on absolute weight of Liver: In group received methanol extract of Origanum majorana leaves (200mg/kg) the absolute weight of liver remained close to the absolute weight of liver in control group. Disease control group increased in liver weight comparatively to control group. The liver weight of treated group with extract was restored with treatment of plant extract. Administration of isolated compound (Hesperetin) was significantly ($P<0.01$) restored the liver weight of treated group similar to standard treated group.

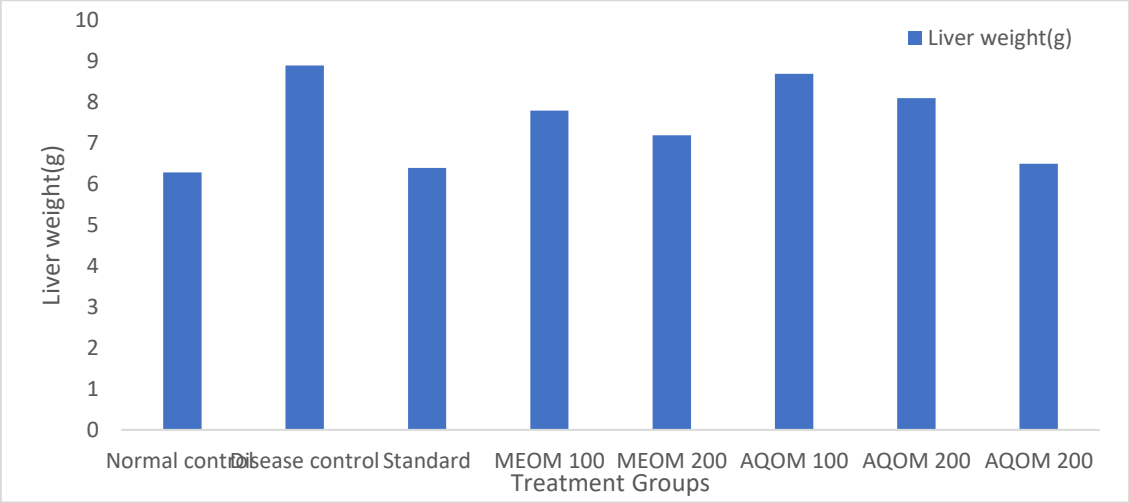


Figure 6.30: Effect of Origanum majorana leaves extract on absolute weight of Liver

Effect on the MDA content and SOD level in liver:

The difference between malondialdehyde content in normal and disease control was quite significant. Maximum recovery was shown by the group that received 200 mg/kg of methanol extract of Origanum majorana leaves. In group received methanol extract of Origanum majorana leaves (200mg/kg) the SOD level remained close to control group. The SOD level of treated group with extract was restored with treatment of plant extract. Isolated compound (Hesperetin) treated group showed SOD level similar to standard treated group

This is suggestive of the fact that supplementation of diet with any drug increases lipid peroxidation, and can be lowered by the methanol extract of Origanum majorana leaves

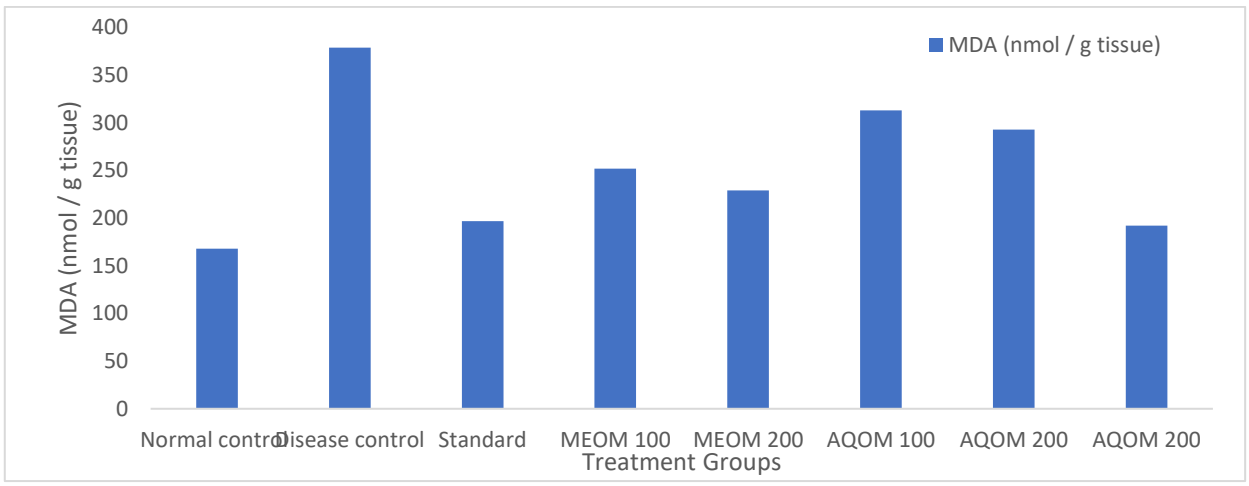


Figure 6.32: Effect of Origanum majorana leaves extract on MDA contents in liver of animals

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 2252-2259 | ISSN:2251-6727

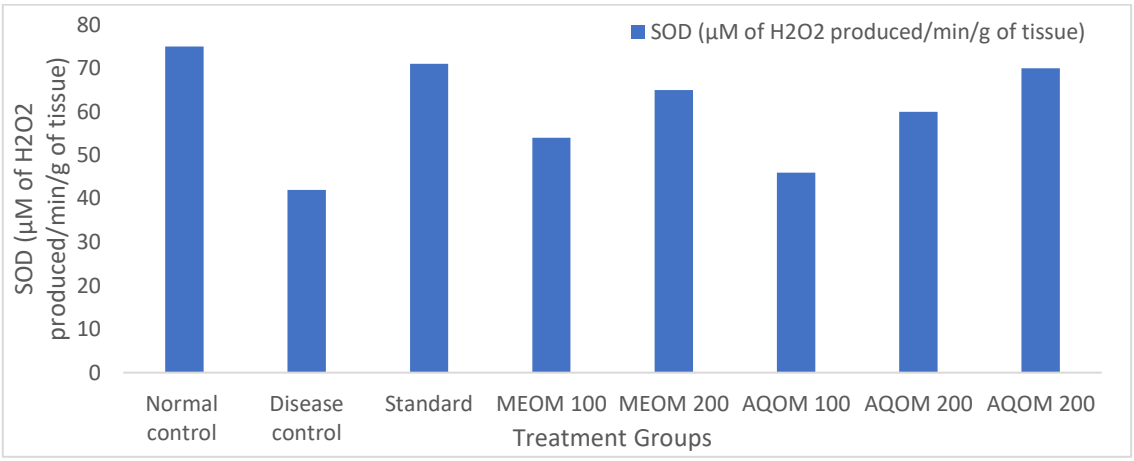


Figure 6.34: Effect of Origanum majorana leaves extract on SOD level in liver of animals

Damage to the structural integrity of liver is reflected by an increase in the levels of serum transaminases AST, ALT and ALP, because they are cytoplasmic in location and are released into the circulation after cellular damage. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP, bilirubin which are originally present in the cytoplasm

When there is hepatopathy, these enzymes and molecules leak into the blood stream, which serves as an indicator for the liver damage. The abnormally high levels of serum ALT, AST, ALP, total bilirubin our study are the consequence of paracetamol-induced liver dysfunction and denotes the damage to the hepatic cells. The rise in ALT activity is almost always due to hepatocellular damage and is usually accompanied by rise in AST. High levels of AST indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury; ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner.

Maximum recovery was shown by the group that received 200 mg/kg of methanol extract of Origanum majorana leaves. In group received methanol extract of Origanum majorana leaves (200mg/kg) the biochemical parameter remained close to control group. Isolated compound (Hesperetin) treated group showed biochemical parameter similar to standard treated group

Conclusion

Paracetamol, used widely as antipyretic and analgesic drug, produces acute toxic effect at high doses, which leads to liver damage. Liver is an important organ and a central one for many of the metabolic functions of the body, decomposition of toxic and waste substances, and disposal of harmful substances from the body. Liver illnesses are still the serious problem of human health. Making active oxygen species is an unavoidable result in aerobic organisms, and their removal is done at a basic level and with regard to the useful physiological functions and their harmful effects. This sensitive balance is for retaining the intracellular redox state which has an important role in optimizing cell functions. Excessive accumulation of free radicals or the body's inability to remove them causes the relocation of redox equilibrium for creating oxidation states in the body.

Group administered with hepatoprotective compound silymarin and isolated compound (Hesperetin) showed biochemical parameter similar to standard treated group. Treatment of animals with methanol extract of Gardenia gummifera as well as isolated compound (Hesperetin) provide protection against paracetamol induced hepatotoxicity.

REFERENCES

[1] Ajith TA, Sheena N, Janardhanan KK. Phellinus rimosus protects carbon tetrachloride induced chronic hepatotoxicity in rats: antioxidant defense mechanism. Pharmaceut Biol. 2006;44:467–74.

[2] Yoshikawa T, Tanaka H, Yoshida N, Seto O, Sugino N, Kondo N. Adjuvant arthritis and lipid peroxide protection by superoxide dismutase. Lipid Peroxide Res. 1983;7:108–10.

[3] Yuda Y, Tanaka J, Hirano F, Igarani K, Satch T. Participation of lipid peroxidation in rat pertussis

vaccine pleurisy. Chem Pharm Bull. 1991;39:50 5–6.

[4] Coskun O, Yakan B, Oztas E, Sezen S, Gunaydin A. Antioxidant and hepatoprotective activity of Vitamin E and Egb 761 in experimental endotoxemic rats. Int J Med Sci. 2000;30:427–32.

[5] Weisman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory diseases and progression to cancer. Biochem J.1996;313:17–29.

[6] Lin CC, Yen MH, Lo TS, Lin JN. Evaluation of hepatoprotective and antioxidant activity of Boehmeria nivea var. nivea and B. nivea var. tenacissima. J Ethnopharmacol.1997;60:9–17.

[7] Charles DJ. Marjoram sweet. In:Antioxidant Properties of Spices, Herbs and other Sources. New York, Springer; 2013:393-399.

[8] 2. Lis A, Piter S, Gora J. A comparative study on the content and chemical composition of essential oils in commercial aromatic seasonings.Herba Polonica. 2007;53:21-26.

[9] Nahida J, Wissal D, Thouraya, et al. Essential oil composition of Origanum majoranaleaves. INIST-CNRS. 2007;1:190-193.

[10] Aghili MH. In: Rahimi R, Shams Ardekani MR, Farjadmand F, eds.Makhzan-al-Advia. Tehran, Iran: Tehran University of Medical Sciences; 2009:735.

[11] Tonkaboni MM. In: Rahimi R, Shams Ardekani MR, Farjadmand F, eds.Tohfeh-al-Momenin. Tehran, Iran: Shahid Beheshti University of Medical Sciences; 2007:290-291.