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RESEARCH ARTICLE
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AMELIORATIVE EFFECT OF PEPPERMINT OIL (*MENTHA PIPERITA L.)* IN LIVER FUNCTION AND SERUM LIPID PEROXIDATION BIOMARKERS IN MALE ALBINO RATS WITH INDUCED NONALCOHOLIC FATTY LIVER DISEASE: A RANDOMIZED CLINICAL CONTROL TRAIL

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Abstract

The complexity, diagnosing challenges, and lack of recognized therapies for non-alcoholic fatty liver disease have made it a major problem. It is anticipated that non-alcoholic fatty liver disease (NAFLD) will surpass hepatitis C as the most prevalent chronic liver disease in adults and children over the next ten years. It is also anticipated to emerge as the primary reason for liver transplantation. It coexists with obesity, hypertension, type 2 diabetes mellitus, and dyslipidemia. Current clinical trials have concentrated on a number of disease reasons, and how these treatments fit into the current paradigm of substrate overload lipotoxic liver injury has been addressed here. Many of the approaches concentrate on downstream events such inflammation, damage, and fibro genesis. Many natural therapies are used to combat elevated liver function enzymes and lipid peroxidation in patients of NAFLD. Mentha piperita L. and M. arvensis var. piperascens, two perennial plants in the Labiatae family, are used to extract peppermint oil from their leaves. Popular and significant, this plant is used extensively for a variety of therapeutic purposes in many indigenous medical systems. These include analgesic, anaesthetic, antiseptic, astringent, carminative, decongestant, expectorant, nervine, stimulant, stomachic, inflammatory diseases, ulcer, and stomach issues. The present study was to highlight the therapeutic effect of peppermint oil against elevated liver enzymes alanine transaminase (ALT) aspartate transaminase,(AST) and melanodialdehyde(MDA).For this purpose the paper mint oil was firstly observed for its chemical composition afterwards Thirty (30)male albino rats were induced with induced fatty liver disease by induction of using the mixture of high fat and high fructose diet and divide them in equal quantity in equal groups in which control group was G0 was observed as control group was not received and G1 and G2 were observed as treatment group and they were given 30ml and 60 ml of peppermint oil as per kg of their body weight. After that liver

enzymes ALT, AST AND MD were measured before and after the trial using ANOVA test. Both treatment groups showed significant reduction in elevated liver enzymes alanine transaminase, and aspartate transaminase. A significant reduction was also seen in lipid peroxidation parameter melanodialdehyde in treatment groups. All results were taken significant art $p<0.05$.

Keywords: Peppermint oil, *Mentha piperita L.*, menthol, liver function enzymes, oxidative stress, lipid peroxidation

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), which affects over 25% of adults globally, has been the most common chronic liver ailment during the past 40 years (Younossi, Zobair M., et al., 2016). NAFLD is known to have a close, reciprocal relationship with several elements of the metabolic syndrome (Zobair et al., 2018) The incidence of non-alcoholic fatty liver disease (NAFLD) varies between 135% and 318% throughout Africa and the Middle East1, suggesting variations in total caloric intake, physical activity, distribution of body fat, socioeconomic level, and genetic makeup (EASO, 2016). According to Ye, Qing, et al. (2020), NAFLD is presently the most prevalent cause of chronic liver disease globally. NAFLD affects up to 80% of obese people and 473-637% of type 2 diabetic patients. These people are typically obese or have additional metabolic risk factors.Fatty liver also develops in people who consume more of the bakery and fine flour foods. (Lonardo et al., 2017). NAFLD, also known as non-obese or lean NAFLD, can occur in some people with a healthy BMI (for example, 25 kg/m2 in White people and 23 kg/m2 in Asian people), due to its close relationship with the metabolic syndrome (Polezos, Stergios A., 2019). The goal of research over the past few decades has been to determine if the AST:ALT ratio may be utilized to differentiate non-alcoholic fatty liver disease (NAFLD) spectrum from alcoholic liver disease (ALD).

Vitamin B6, pyridoxal-5'-phosphate, is necessary for the proper function of the AST and ALT enzymes. Its absence causes the AST:ALT ratio to increase because it has a significantly higher impact on ALT production than it does on AST production in nutritionally deficient heavy drinkers. (Diehl, Anna Mae, et al., 1989; Cohen, JA., 1979).

The purpose of liver enzyme analysis in non-alcoholic fatty liver disease (NAFLD) is to stratify future risk and detect and change related metabolic risk factors, such as hypertension, hyperlipidemia, and glycemic control, at an early stage. (Stephen A. Harrison and others, 2018). Currently, a large number of populations are taking herbal medicine and naturally existing solution to treat their diseases. Trsolutionsl Chinese and Japanese medicine also promotes the use of herbal solutions for diseases. However, with the increase in the use of herbal medicine, some regions of world have reported increase in cases with side effects of herbal medicines (Murag et al., 2021,).

Numerous studies have shown that a decrease in chronic illnesses including DNA damage, mutagenesis, and carcinogenesis is associated with the presence of natural antioxidants derived from a variety of aromatic and medicinal plants (Reddy et al., 2003). Peppermint oil contains many therapeutic components including menthol as its major constituent. Menthol being its major component can be effective in liver cell injury and diseases. It can be helpful in management of liver enzyme functions and also in preventing the lipid peroxidation (Çoban & Baydar, 2016). Because they pose less of a threat to the environment and the health of mammals, alternative antioxidant active substances including plant extracts and essential oils are attracting increasing attention in study. Phenolic chemicals, including flavonoids, are among the most significant classes of antioxidants found in nature (Pietta, 1998). Research indicates that peppermint possesses a high level of antioxidant activity (Atanassova et al., 2011).

NOVELTY OF RESEARCH

The study mainly focused on therapeutic effect of peppermint oil against elevated ALT, AST, and MDA in induced nonalcoholic fatty liver disease in male albino rats. Firstly it was evaluated by its chemical characterization to check its bioactive ingredients for the improvement of liver function

enzymes like ALT, AST and the lipid peroxidation biomarkers to reduce the complications of CVDs and related metabolic disorders in order to protect the people from the usage of conventional medicines that are leading to cause major complications

MATERIAL AND METHODS

Peppermint oil that was 100 percent pure was bought from Chiltanpure in Pakistan. The peppermint oil was diluted with regular saline. Using an ELISA kit that was acquired from a Swedish manufacturer, the levels of ALT, AST, and MDA were determined. The university lab had easy access to chloroform, which was used to sedate animals.

PROCUREMENT AND HANDLING OF ANIMALS

18 male wistar albino rats of 12 weeks, and 220-235gm weight were purchased from National Institute of Health (NIH), Islamabad. Rats were kept in glass cages in an animal House and were fed with *ad libitum* water and deep. 12- Hours light and dark cycle was maintained at animal house.

INDUCTION OF DISEASE

For the induction nonalcoholic fatty liver disease high fat-high fructose diet i.e., 50% fat diet and fructose in water was given to rats for four weeks. After four week, rats went through ultrasonography to confirm the development of nonalcoholic fatty liver disease (Barrière et al., 2018).

PREPARATION OF PEPPERMINT OIL DILUTION.

Peppermint oil was diluted by using normal saline and was given intravenously to rats.

Treatment Groups and Treatment Plan

30 rats were divided into three groups i.e., group 1; rats with no treatment was on their normal low fat diet excluding the paper mint oil and group 2 G1; rats with NAFLD which received 30ml/kg of body weight of peppermint oil and group 3 which received 60ml/kg of the body weight of peppermint oil. Dose was given orally with the help of catheter (Marjani et al., 2012).

Table I: Treatment-Plan

Treatment Groups	Subjects	Treatment
G_0-Control group	10	No treatment
G_1-Treatment group-1	10	30ml/kg of body weight of peppermint oil
G_2-Treatment group-2	10	60ml/kg of the body weight of peppermint oil

SAMPLE COLLECTION AND ANALYSES

Chloroform was used to anesthetize rats, and then blood sample was drawn before and at the end of trial after administration peppermint oil in rats. (Manivannan, R., and R. Shopna., 2017)

STATISTICAL ANALYSIS

The degree of significance ($p<0.05$) was investigated utilizing descriptive statistical analysis employing one way ANOVA followed by a two-sample t-test to compare the means of before and after the dosage under a Completely Randomized Design (CRD). The shown results mean ± standard deviation. IBM SPSS Statistics 20 is used for all statistical studies (D'Agostino, 2017).

RESULTS

This research study was designed to investigate the therapeutic effect of peppermint oil in two different doses in induced NAFLD and results were compared between the 0-day and 60^{th} day in control group and treatment group.

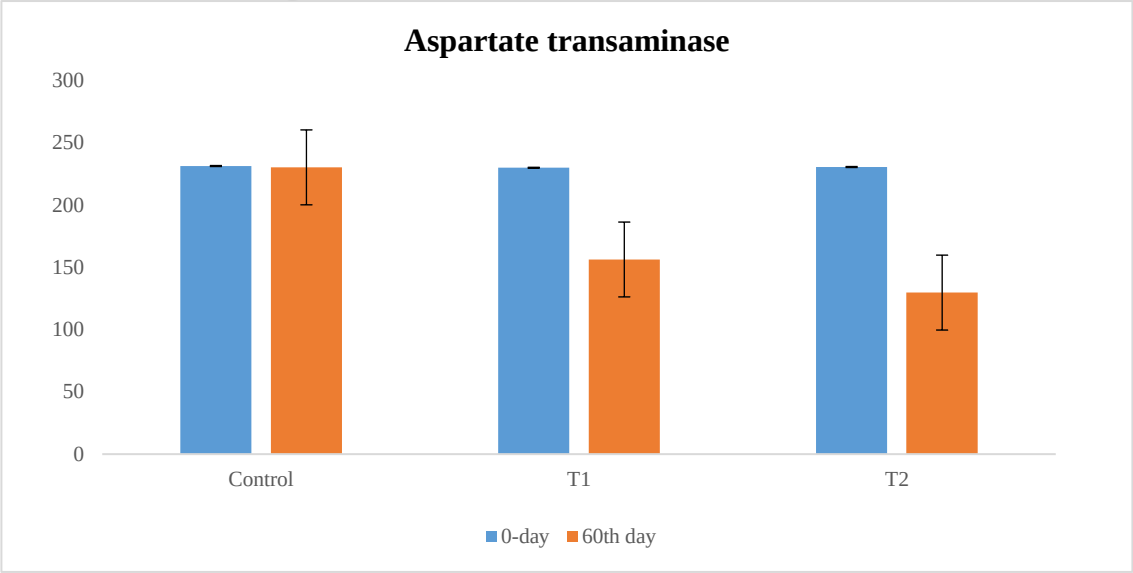
In Figures I, II, and III, serum MDA, ALT, and AST are measured before and after the trail on days 1 through 60 using 30 mg/kg and 60 mg/kg of peppermint oil, respectively. The treatment group's

levels of MDA, ALT, and AST liver enzyme analyses were all considerably lower than those of the control group.

EFFECT OF PEPPERMINT OIL ON AST LEVEL.

Figure 1 illustrates the considerable ($p<0.05$) decrease in AST levels in the treatment group following oral administration of peppermint oil. Every result was considered significant at $p<0.05$.

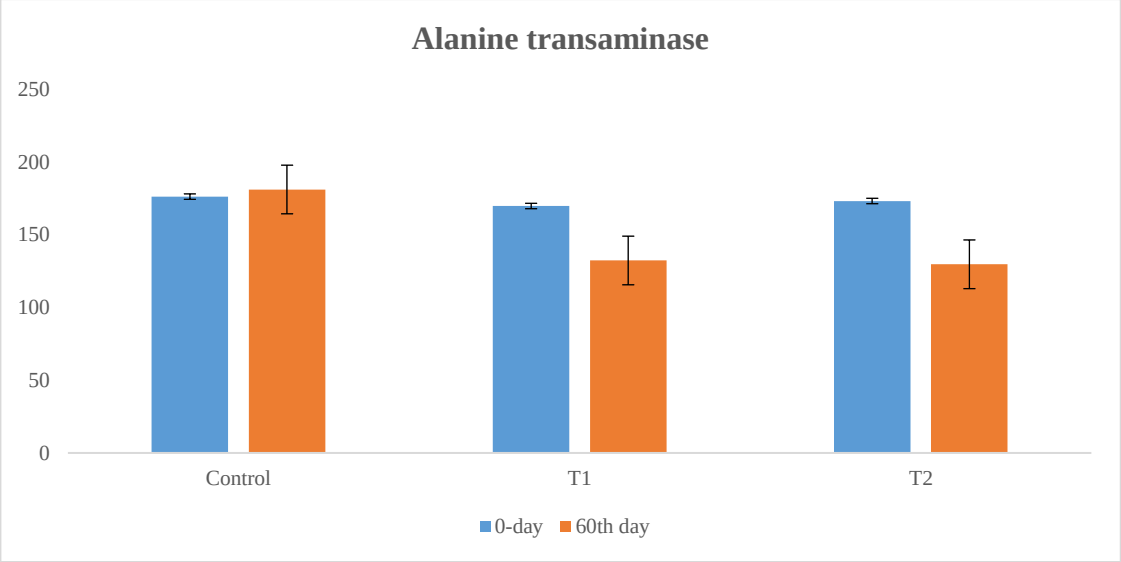
Fig 1. Mean ± S.D for serum AST levels IU/L.



Effect of Peppermint oil on ALT Level.

Figure 2 illustrates the considerable ($p<0.05$) reduction in ALT levels seen in the treatment group following the delivery of peppermint oil. Every result was considered significant at $p<0.05$.

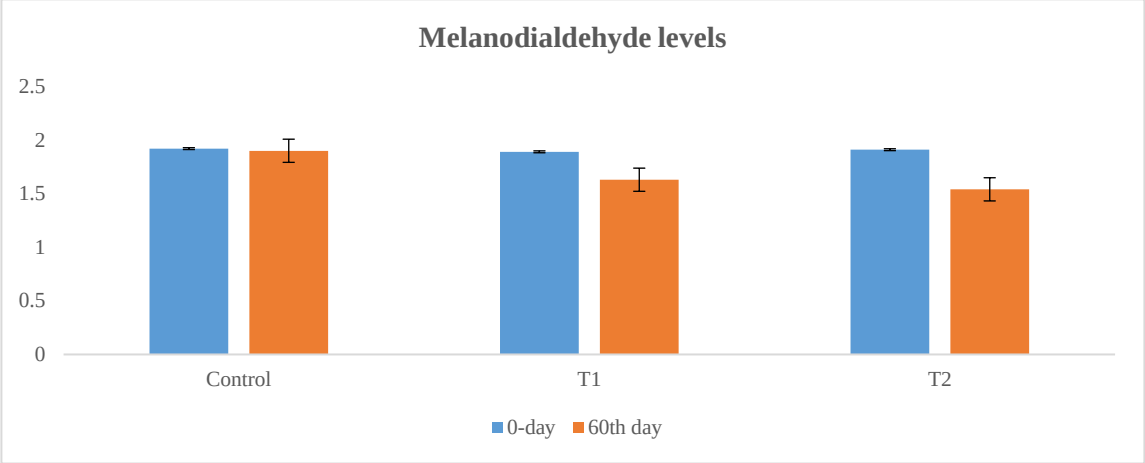
Fig 2. Mean ± S.D for ALT levels in IU/L.



Effect of Peppermint Oil on Malondialdehyde (MDA) levels

Figure 3 illustrates the results, which indicated a substantial ($p<0.05$) decrease in MDA levels in the treatment group following oral peppermint oil delivery. In therapy group 2, the decrease in serum MDA levels is more pronounced. Every result was considered significant at $p<0.05$.

Fig 3. Mean ± S.D for MDA levels in nmol/ml.



DISCUSSIONS

The results of this study indicate that during the metabolism of peppermint oil, MDA generation was decreased to the point where elevations in AST and ALT were also noted. The purpose of the study was to ascertain if peppermint oil might treat male albino rats with induced NAFLD. To assess the liver functioning serum level of ALT and AST were measured before and after the trial. To determine the effect of peppermint oil on lipid peroxidation serum MDA levels were measured. Results of the study reported the reduction in ALT, AST and MDA levels in response to the two different doses of pepper mint oil. These findings were like the findings of another study which also reported reduction in ALT, AST, and MDA levels in rats with and without immobility stress. Study reported that peppermint oil contain menthol which reduce the oxidative stress in the body and thus helped in preventing lipid peroxidation (Marjani et al., 2012). Mimica-Dukic et al. revealed in another investigation that M. piperita oil had a potent ability to scavenge free radicals. M. piperita inhibited the OH radical's ability to form in their experiment and lowered the radical generator 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by 50%. Rats given daily oral doses of 83 L/kg peppermint oil for 28 days had substantially greater ALP levels but no increase in GGT or ALT when compared to the control group. Antioxidant potential of menthol of peppermint oil also proved beneficial in lowering the lipid peroxidation by scavenging free radicals in the body. Menthol showed lipid peroxidation preventive ability is cardiovascular diseases (Cantanhêde et al., 2021). Menthol supplied by gavage to rats at 200, 400, and 800 mg/kg for 28 days dramatically increased absolute and relative liver weights, as well as hepatocyte vacuolization at all doses, with no sign of encephalopathy (Thorup, I., et al. 1983) Hepatocytes protective effect of peppermint oil is also reported in another study that discussed the effect of peppermint oil on liver function impairment induced due to the consumption of repeated fried oil based foods. The study reported that peppermint oil alone and in combination with thyme oil not only reduced the oxidative stress in the body but helped in preventing the oxidative stress induced damage to the hepatocytes. Study also reported that peppermint oil alone and in combination with thyme oil improves liver functioning by regulating the action of ALT and AST in the body. Study explained that peppermint oil is also effective oxidative stress induced cardiovascular diseases due to the consumption of repeated fried oil in rats (Balamash & Nour, 2019). Similarly, in another study, peppermint oil showed ameliorative effect on liver function enzymes which showed impaired functioning due to the hepatorenal toxicity induced due to the use of anti-inflammatory drugs. Peppermint oil not only regulated the liver enzymes function but also helped in reducing the toxicity of drug in liver and kidney (Al-Hamdany et al., 2022).

CONCLUSION

Peppermint oil is used externally and by oral administration against many diseases. It is an important part of many pharmaceutical preparation which are helpful in lowering the severity of disease.

Peppermint oil has antibacterial, anti-inflammatory, fungicidal, anti-toxic, mosquito repellant properties. Study concluded hepato-protective effect of peppermint oil. It not only regulated the liver functioning of enzymes but also helped in lowering the oxidative stress. It reduced the oxidation of lipid by scavenging free radicals in the body. It is likely possible to lessen the impact of immobilization stress on liver enzymes by using an effective amount of peppermint oil. To fully understand the process of stress-dependent free radical formation and how it affects oxidative changes in various tissues, more research is required.

Conflict of Interest

There was no conflict of interest among the authors of the study.

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