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Hepatoprotective Effects of Ethanolic Extract of *Mentha Piperita* (Peppermint) Leaves on Mercury Chloride-induced Damage in Adult Rats

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ABSTRACT

This research aimed to assess the potential effect of mint leaf as ameliorative approach in mercury chloride-induced hepatotoxicity in adult rats. 35 adult rats were randomly assigned to 7 groups, each consisting of 4 animals. Groups A and B received 5mg/kg and 0.5mg/kg of mercury chloride (HgCl2) respectively, group C served as the control, while groups D and E received 800mg/kg and 1600mg/kg of Mentha piperita ethanolic leaf extract respectively while groups G and H received 800mg/kg + 5 mg/kg and 1600mg/kg + 0.5mg/kg of ethanoic leaf extract of Mentha piperita and HgCl2 respectively, for 4 weeks, Twenty-four hours after the final treatment, the rats were weighed and sacrificed under ketamine, and their blood which was collected through ocular puncture and centrifuged to get serum. The liver was harvested, fixed in formalin for histological examination. Data was collected such as body weight differences, organ weight, Alanine transaminase level (ALT), Aspartate aminotransferase level (AST) and Alkaline phosphatase level (ALP), RESULT: Decreased ALP levels, Decreased ALT levels (except Group H), Increased AST levels. Combination therapy with EMP and low-dose mercury chloride showed potential benefits. The Histological results showed Group D 800 mg/kg: Mild hepatocellular damage, reduced inflammation, Group E (1600 mg/kg): Minimal hepatocellular damage, near-normal liver architecture while Group G&H Combination therapy (0.5 mg/kg HgCI +800/1600 mg/kg EMP): Significant reduction in liver damage. With the result of the current study, Ethanolic leaf extract of Mentha piperita exhibits hepatoprotective effects against mercury chloride-induced toxicity, suggesting potential therapeutic applications.

BACKGROUND TO THE STUDY

Mercury, a potent neurotoxin, poses significant health risks to humans and animals due to its detrimental effects on the central nervous system. Exposure to mercury can occur through various sources, including contaminated water, food, and occupational exposure, leading to impairments in motor coordination and cognitive performance (Clarkson *et al.*, 2017). The World Health Organisation (WHO) has identified mercury as one of the top ten chemicals of major public health concern, highlighting the need for comprehensive research on its neurotoxic effects. Mercury can be found in three primary states: inorganic, organic, and metallic. Inorganic mercury compounds, such as mercuric chloride, mercurous chloride, and mercurous oxide, can exist in either a mercuric (Hg Il) or mercurous (Hg1) state and pose potential harm to humans (Clark *et al.*, 2019). Mercury is released into the environment

through human activities such as mining, smelting, and industrial processes (UNEP, 2019). Mercury-containing products, such as thermometers and fluorescent light bulbs, also contribute to environmental contamination (EPA, 2020).

Mercury exposure has been linked to various health problems, including neurological damage, developmental delays, and cardiovascular disease (WHO, 2017). Methylmercury, in particular, is a potent neurotoxin that can cause permanent damage to the brain and nervous system (Grandjean *et al.,* 2014). Mercury toxicity has been associated with a range of neurological symptoms, including impaired motor function, cognitive deficits, and behavioural abnormalities (Farina *et al.,* 2011). Neurotoxicity studies have shown that mercury exposure can induce oxidative stress, inflammation, and mitochondrial dysfunction in the brain, leading to neuronal damage and dysfunction (Farina *et al.,* 2011; Franco *et al.,* 2016). The developing brain is particularly vulnerable to mercury's neurotoxic effects, with prenatal exposure linked to cognitive and motor impairments in children (Landrigan *et al.,* 2016).

Mentha piperita, commonly known as peppermint, is a widely used herbal remedy for various health ailments (Blumberg *et al.,* 2016). The leaves of the plant contain essential oils, flavonoids, and phenolic acids, which contribute to its therapeutic properties (Kumar *et al*., 2011). Mentha piperita has been reported to possess Antioxidant activity: Protects against oxidative stress and cell damage (Samarth *et al.,* 2012). Anti-inflammatory activity: Reduces inflammation and alleviates pain (Shukla *et al.,* 2012). Antimicrobial activity: Exhibits antibacterial and antifungal properties (Mimica-Dukic *et al*., 2014).

Mentha piperita has been traditionally used for digestive issues: Relieves symptoms of irritable bowel syndrome (IBS) and indigestion (Cappello *et al.,* 2017). Respiratory issues: Eases congestion and coughs (Sharma *et al.,* 2011). Neurological disorders: May help alleviate anxiety and stress (Kumar *et al.,* 2013). Mentha piperita is regarded as a soothing herb with a longstanding history of use and has been employed for centuries to alleviate stomach discomfort and indigestion (Carney *et al.,* 2011). According to a 2019 analysis, clinical trials comparing peppermint oil to placebo support its efficacy in managing various gastrointestinal issues, such as indigestion, irritable bowel syndrome (IBS), paediatric stomach pain, and postoperative nausea. Researchers note that mint's mechanisms include combating harmful microbes, regulating muscle relaxation, and mitigating inflammation. Another review from the same year, examining 12 randomised controlled trials, concluded that peppermint oil is both safe and effective in alleviating pain symptoms among adults with IBS.

However, a separate 2019 study involving 190 individuals with IBS, conducted as a randomised, double-blind trial, revealed that peppermint oil failed to produce a significant reduction in symptoms (Bray *et al.,* 2023).

The liver, the largest internal organ, plays a vital role in maintaining overall health (Renz *et al.,* 2014). Its functions include detoxification, metabolism, bile production, glycogen storage, filtration, hormone regulation, protein synthesis, and immune support (Kinkhabwala *et al.,* 2014). The liver is essential for energy production, nutrient absorption, infection protection, maintaining healthy skin, hair, and nails, and supporting mental health (Guyton *et al.,* 2016).

However, liver damage can lead to impaired detoxification, nutrient deficiencies, increased infection risk, skin problems, and mental health issues (Kumar *et al., 2017).* The liver's critical role necessitates attention to its health, highlighting the importance of understanding liver diseases, promoting liver wellness, and recognising the consequences of liver damage (Lee *et al.,* 2019).

METHODS

Experimental Animals

35 adult male Wistar rats, were procured from a private animal farm in Nnewi, Anambra State. The rats were housed in a well ventilated stainless-steel cages and adapted to the laboratory environment in the Department of Anatomy within the period of acclimatization. Beddings made of wood shavings, obtained from a sawmill, were replaced everyday throughout the experiment. The animals were maintained under standard conditions, including a temperature range of 25-28°C, relative humidity of 60-80%, and a 12-hour day/night photo period. All procedures involving the animals were conducted in adherence to the ethical guidelines approved by the Ethical Approval Committee for Animal Care and Use in the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. This compliance follows the "Guide for the Care and Use of Laboratory Animals" established by the National Academy of Sciences and published by the National Institute of Health Guide for the Care and Use of laboratory animals 2011).

Experimental Design

The experiment lasted for a period of 7weeks; three (3) weeks for acclimatization and four weeks of administration of Mercury chloride and ethanolic leaf extract of Mentha piperita and were done using the oral gavage method. The rats were weighed prior to administration and subsequently during the course of the experiment and were grouped as follow;

Group A received 5mg/kg of Mercury chloride once daily for a period of four weeks with animal feed and distilled water ad libitum.

Group B received 0.5mg/kg of Mercury chloride once daily for a period of four weeks with animal feed and distilled water ad libitum.

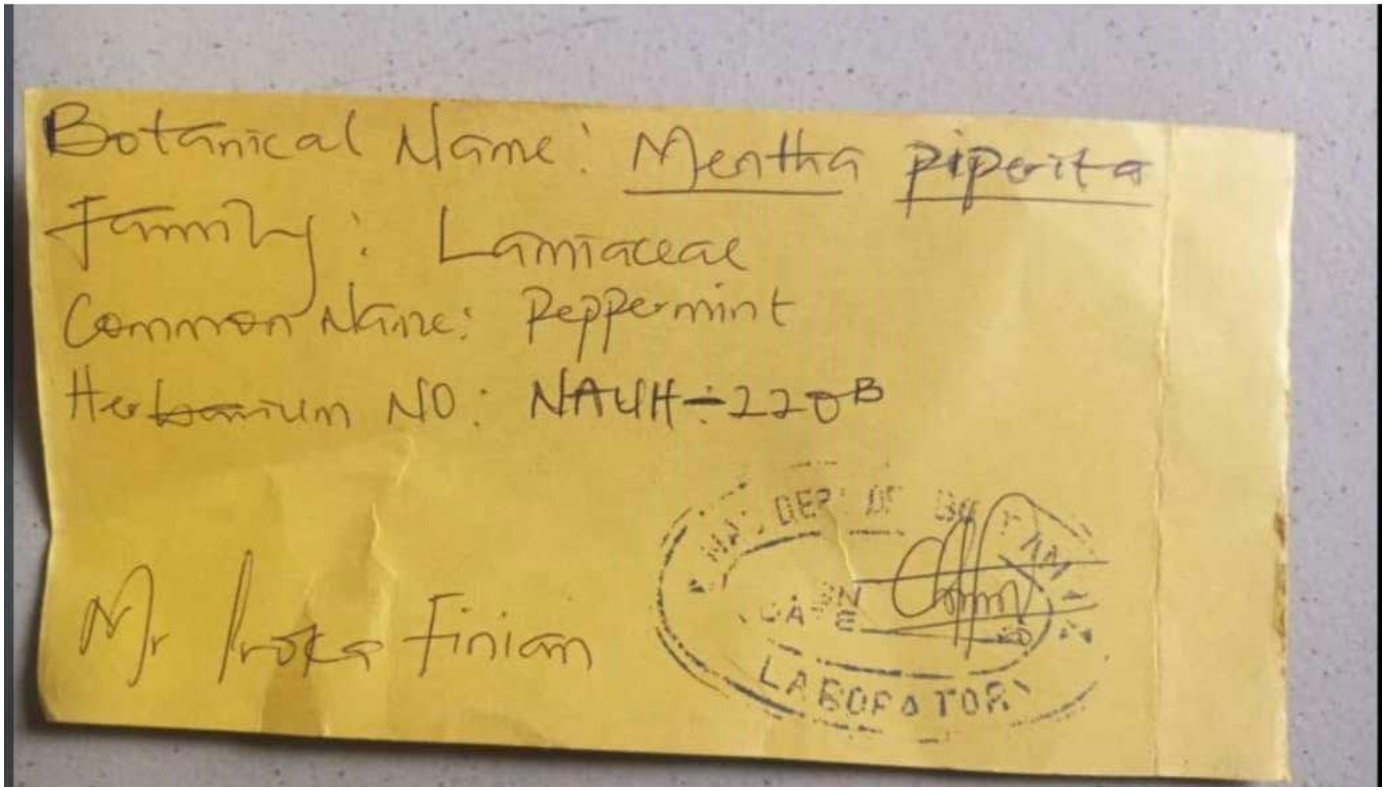
Group C was used as control group and received animal feed and water daily ad libitum throughout the period of experiment.

Group D received 800mg/kg of ethanolic leaf extract of *Mentha piperita* once daily for a period of four weeks with animal feed and distilled water ad libitum.

Group E received 1600mg/kg of ethanolic leaf extract of *Mentha piperita* once daily for a period of four weeks with animal feed and distilled water ad libitum.
Group G received 800mg/kg of ethanolic leaf extract of *Mentha piperita* and 0.5mg/kg of Mercury chloride once daily for a period of four weeks with animal feed and distilled water. Group H received 1600mg/kg of ethanolic leaf extract of *Mentha piperita* and 0.5mg/kg of Mercury chloride once daily for a period of four weeks with animal feed and distilled water.
This study was carried out at the Animal house of the College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Anambra State, Nigeria, Ethical approval was sort and obtained from the Ethical committee, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus.

Collection of *Mentha piperita* leaf

Mentha piperita leaf (*peppermint*), was obtained from farm in Jos, Plateau state , Nigeria and transported in well ventilated sack bags to the College of health
Sciences Okofia, Nnewi, Anambra state. Fresh leaves of *Mentha piperita* were collected for identification and authentication by a botanist from a Botany Department of Nnamdi Azikiwe University, Awka. The leaves were thoroughly washed with clean running water to remove dirt and soil. The leaves were separated and air dried. The dried plant material was powdered using a heavy duty blender.
250g of the grinded *Mentha piperita* leaf was mascerated in 1000mls of 98% absolute ethanol (BDHEngland) for 48hours under mechanical sheker (Uniscope101). The mixture was sieved after 48hours using porcelain cloth, and was further filtered using whatmann no1 filter paper into a clean glass beaker.
The filtrate was concentrated using Digital Rotary Evaporator (TT-55 Technical and Technical USA) and was further dried using thermostat oven (DHG 9023A PEC Medicals USA) into a pastelike form and stored in a Nexus refridgerator for further usage.The method followed the procedure described by (Attar and Zaid *et al.,* 2012).



Phytochemical Analyses of *Mentha piperita* Leaf.
This was done by the Department of Biochemistry in the Nnamdi Azikiwe University, Nnewi Campus. 0.2g of sample was weighed and transferred in a test tube and 15ml of Methanol was added. The test tube was allowed to react in a water bath at 600c for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis (Buss *et al.,*2010).

Quantification by Gas Chromatography (GC)

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization

system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliterof the prepared 1% of the extracts diluted with respective solvents was injected in ansplitless mode. Relative quantity of the chemical compounds present in each of the extracts of Mentha piperita was expressed as percentage based on peak area produced in the chromatogram (Kelly and Nelson *et al.,*2014).

Identification of chemical constituents

Bioactive compounds extracted from different extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Table 2 Phytochemical Components of *Mentha piperita*

Identification	CONC (ug/ml)
Kaempferol	3.33
Steroid	8.29
Proanthocyanidin	10.49
Anthocyanin	2.07
Narigenin	7.43
Dihydrocytisine	5.91
Cyanogenic glycosides	20.19
Ammodendrine	22.07
Tannin	12
Flavonones	8.74
Cardiac glycoside	4.01
Flavones	5.72
Ribalinidine	0
Phytic acid	8.81
Spartein	15.97
Oxalate	1.31
Aphyllidine	4.24
Epihedrine	3.94
Sapogenin	11

Acute toxicity (LD_{50}) for *Mentha piperita*

The LD_{50} of the Ethanolic leaf extract of Menthapeperita was determined using a new method for determining acute toxicity in animal models as described by (Enegide *et al.,* 2013). This procedure is carried out in three stages with the outcome of each stage determining whether to terminate or move on to the next stage. The total of 10 male wistar rats were used. The initial stage, which is stage one require four rats. The extract was administered to the rats orally. The rats were grouped into four groups of one rat each. Rat in group one was administered 10 mg/kg/bw of the extract, while those in groups two, three and four were each administered 100, 300 and 600 mg/kg/bw of the extract respectively. Since no mortality and signs of toxicity were observed, another three groups of rats with one rat in each group were administered 1000, 1,500 and 2,000 mg/kgbw (stage 2). Again no mortality and signs of toxicity were observed. Based on this, another three groups of rats with one rat in each group were administered 3,000, 4,000 and 5,000 mg/kgbw of the extract respectively (stage 3). There was no mortality observed at 5,000 mg/kgbw. Based on this, a confirmatory test was carried out according to the method described by (Enegide et al., 2013), by administering 5,000 mg/kgbw of the extract to each of two groups of one rat each. The Observation was made on the rats for 1 h after administration and 10 min for every 2 h interval for 24 h.

$$\frac{[M_0+M_1]}{2}$$

Where, M_0= Highest dose of the test substance that gave no mortality = 0

M_1 = Lowest dose of the test substance that gave mortality = 0

$$\frac{\text{No of dead rats}}{n}$$

Where n= number of rats used

Preparation and Administration of Mercury chloride solution

Dry powder of mercury chloride (HgCl2, 99% purity) manufactured by Loba Cheme PVT Ltd, Mumbia,40005,India was purchased from JulimarkEnterprises, Yemetu, Ibadan, Nigeria. 1g of mercury chloride was dissolved in 100ml of distilled water to obtain mercury chloride solution, which was administered to the experimental animals orally using an oral gavage. Dosage was administered based on the rat's body weight.

Termination of Experiment.

24hours after the last administration of ethanolic extract of *mentha piperita* leaf and mercury chloride solution, the rats were sacrificed by cervical dislocation after chloroform sedation which lasted for a period of four weeks, Blood samples were collected through ocular puncture, into a plain blood specimen bottles. The serum was separated using a centrifuge and collected in a plain specimen bottles and stored in a refrigerator for serum liver function test. After collection of blood samples the liver was quickly dissected out, weighed and fixed in 10% formal saline.

Liver Function Test

There are several liver function test. Three main tests will be done for this experiment, they include; Alkaline Phosphatase level (ALP), Aspartate Aminotransferase level (AST) and Alanine transaminase level (ALT). Alkaline Phosphatase Level (ALP) measures the amount of alkaline phosphatase enzyme in the bloodstream which indicate liver inflammation. Aspartate aminotransferase (AST) level is done along side ALT to check for further damages done to the liver. Finally, Alanine transaminase level (ALT is done to check the amount of alanine transaminase that has been released in the blood stream, which indicates liver damage. These test requires a blood sample from the rats.

HEAMATOXYLIN AND EOSIN STAIN

Histological protocol by (Drury and Wallington, 1973) was adopted. Sections were dewaxed using xylene for one (1) minute and then rehydrated by alcohol through descending grades of ethyl alcohol and thereafter washed in distilled water. The sections were stained with heamatoxylene for twenty (20) minutes and differentiated with 2% acid alcohol for two (2) seconds. The acid alcohol was washed off with tap water and the sections passed through running tap water for ten (10) minutes to regain the blue colour. The sections were counterstained in 1% aqueous eosin for thirty (30) seconds and were dehydrated through ascending grades of ethyl alcohol, cleared in xylene and mounted using DPX. After which, the sections were viewed under light microscope.

DATA ANALYSIS

Data was analyzed using SPSS version 27.0.1 software package. Mean and standard deviation was obtained and one way analysis of variance (ANOVA) was used to compare values between groups. Data was expressed as Mean ± Standard Error of Mean (SEM) and was considered statistically significant when $P \leq 0.05$.

RESULTS

Physical Behavioral Changes

At the beginning of the experiment, the rats were apparently healthy and agile with smoothly laid white hairs on their skin, normal skin color and they increased in size. During the three weeks of acclimatization, their stools were normal and they adapted well to their environment. After four weeks of administration, after assessing their feed it was clear that all groups had normal appetite and normal stool but they were experiencing changes in their hair color and some rats lost motor coordination which were mostly occurring in groups that were administered mercury chloride only.

At the end of administration, all the rats were still healthy and agile.

Effect of ethanolic leaf extract of *Mentha piperita* on Body weight following mercury chloride exposed rats

	Initial weight (g)	Final weight (g)	p-value	t-value
	MEAN ±SEM	MEAN±SEM		
Group A (5 mg/kg of HgCl)	135.00 ±2.88	167.60 ±8.29	0.099#	-2.93
Group B (0.5 mg/kg of HgCl)	180.67±12.45	213.10 ±6.88	0.223#	-1.74
Group C (control)	138.67. ±8.19	176.33 ±3.17	0.024*	-6.27
Group D (800 mg/kg of EMP)	165.33 ±7.96	178.33±16.74	0.313#	-1.33
Group E (1600 mg/kg of EMP)	186.67.±12.54	214.67 ±3.84	0.213#	-1.80
Group G (5 mg/kg of HgCl + 800 mg/kg of EMP)	166.00 ±2.31	176.33 ±3.17	0.187#	-1.97
Group H (0.5mg/kg of HgCl + 1600 mg/kg of EMP)	186.67 ±12.54	212.33 ±9.61	0.366#	-1.158

*: significant

#: not significant

SEM: standard error of mean

EMP: ethanolic leaf extract of *Mentha piperita.*

Data was analyzed using t-test and values were considered significant at $p<0.05$

Table 3: Result showed an increase in the mean body weight in groups A, C, D, E, G, and H; group B had a decrease in the mean body weight when the initial weight was compared to the final weight, which indicate significance in group C, while groups A, B, D, E, G, and H had no significant difference.

Effect of ethanolic leaf extract of *Mentha piperita* on relative liver weight in mercury chloride exposed rats

	Relative liver weight (g)
	MEAN ±SEM
Group A (5 mg/kg of HgCl)	4.58 ±0.31
Group B (0.5 mg/kg of HgCl)	3.45 ±0.23
Group C (control)	3.69 ±0.22#
Group D (800 mg/kg of EMP)	2.83 ±0.49#b
Group E (1600 mg/kg of EMP)	3.28 ±0.69#b
Group G (0.5 mg/kg of HgCl + 800 mg/kg of EMP)	4.13 ±0.28#b
Group H (0.5 mg/kg of HgCl + 1600 mg/kg of EMP)	3.35 ±0.27#b
P-value	0.018
F-ratio	3.818

*: significant

#: not significant when comparison was made to B. a: significant, b: not significant when comparison was made to C.

SEM: standard error of mean

EMP: ethanolic leaf extract of *Mentha piperita.*

Table 4: Result revealed an increase in groups C and G (p=0.586, p=0.130), groups D, E, and H (p=0.157, p=0.681, p=0.805) had a decrease compared to B, which had no significant differences.Also, groups D, E, and H (p=0.059, p=0.345, p=0.432) had a decrease in the relative liver weight, and group G (p=0.312) had an increase compared to C, which indicate no significant differences.

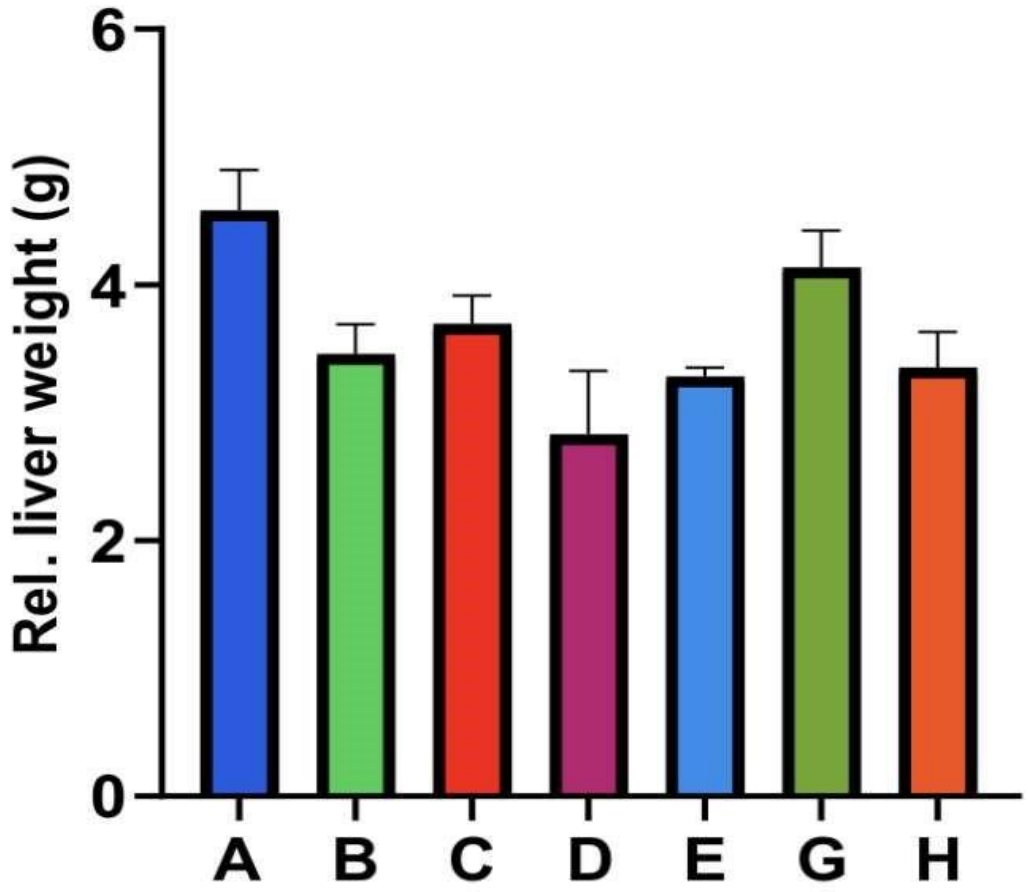


Fig 3: Effect of ethanolic leaf extract of *Mentha piperita* on relative liver weight in mercury chloride exposed rats

4.4. Effect of ethanolic leaf extract of *Mentha piperita* on liver enzymes in mercury chloride exposed rats

*: significant #: not significant when comparison was made to B. a: significant, b: not significant when comparison was made to C.
SEM: standard error of mean
EMLP: ethanolic leaf extract of *Mentha piperita.*

Table 5: result revealed a significant decrease in the mean ALP level in groups D, E, G, and H

	ALP level (IU/L)	ALT level (IU/L)	AST level (IU/L)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A (5 mg/kg of HgCl)	52.33±11.09	11.00±0.56	10.00±1.73
Group B (0.5 mg/kg of HgCl)	40.33±2.90b	12.33±2.91b	10.33±3.28b
Group C (control)	39.33±9.02	13.67±4.25	21.66±9.17
Group D (800 mg/kg of EMP)	4.42±0.28*	5.22±1.25#a	43.98±4.51*a
Group E (1600 mg/kg of EMP)	9.59±1.89*a	6.60±0.85#b	63.43±11.32*a
Group G (0.5 mg/kg of HgCl + 800 mg/kg of EMP)	11.73±1.82*a	12.42±2.61#b	70.33±6.77*a
Group H (0.5 mg/kg of HgCl + 1600 mg/kg of EMP)	14.85±1.03*a	12.65±2.03#b	1.96±9.23*a
P-value	0.000	0.153	0.000
F-ratio	11.381	1.889	16.358

(p=0.000, p=0.002, p=0.003, p=0.006) compared to B. Also, groups D, E, G, and H (p=0.000, p=0.002, p=0.003, p=0.006) had a significant decrease and group B (p=1.000) had an increase compared to C, which had no significance.

The ALT result showed a decrease in groups B, D, E, G, and H (p=0.700, p=0.026, p=0.056, p=0.719, p=0.770) compared to group C, which had significance in groups D, groups B, E, G, and H had no significance. However, groups D and E (p=0.054, p=0.113) had a decrease and groups G and H (p=0.979, p=0.925) had an increase compared to group B, which had no significance.
Serum mean AST results showed a significant increase in groups D, E, G and H (p=0.006, p=0.000, p=0.000, p=0.001) compared to B. Also, groups D, E, G, and H (p=0.05, p=.001, p=0.000, p=0.000) had a significant increase, group B (p=0.294) had an insignificant decrease compared to group C.

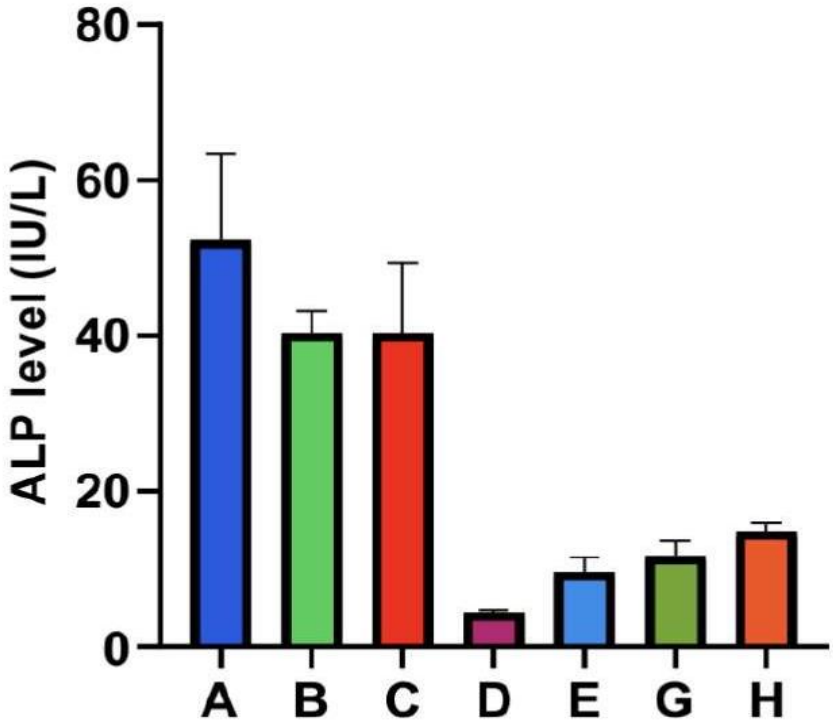


Fig 4: Effect of ethanolic leaf extract of *Mentha piperita* on ALP level in mercury chloride exposed rats

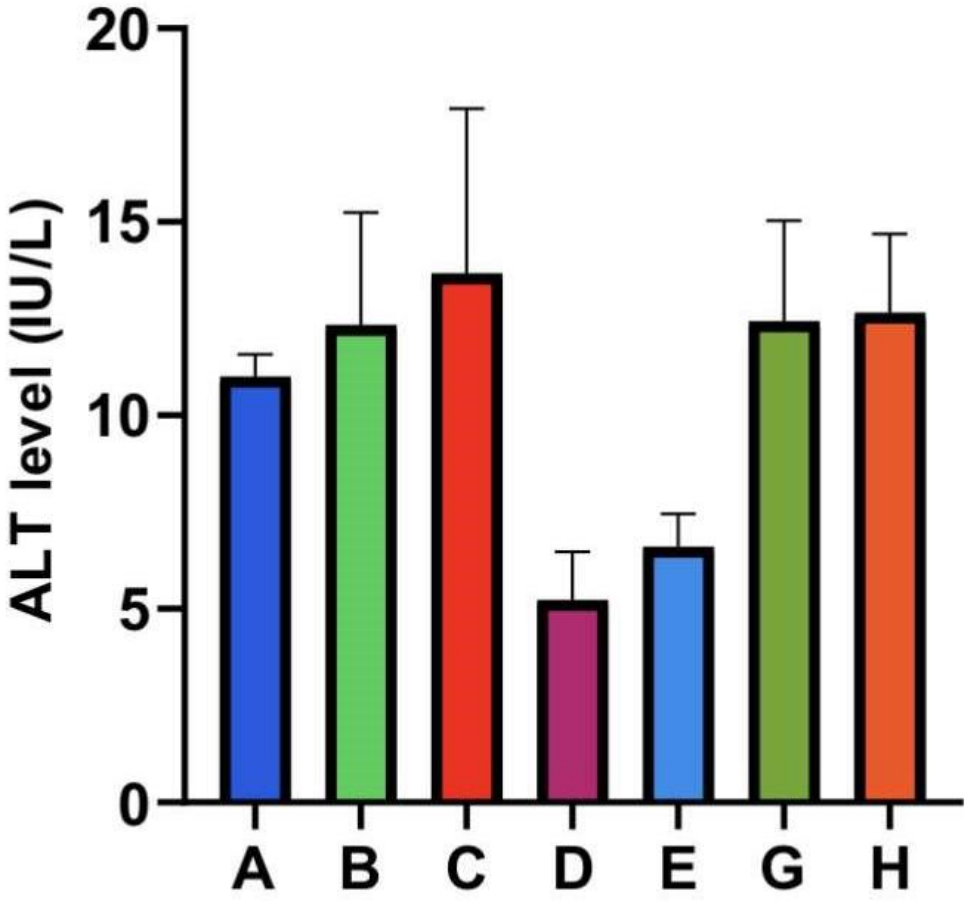


Fig 5: Effect of ethanolic leaf extract of *Mentha piperita* on ALT level in mercury chloride exposed rats.

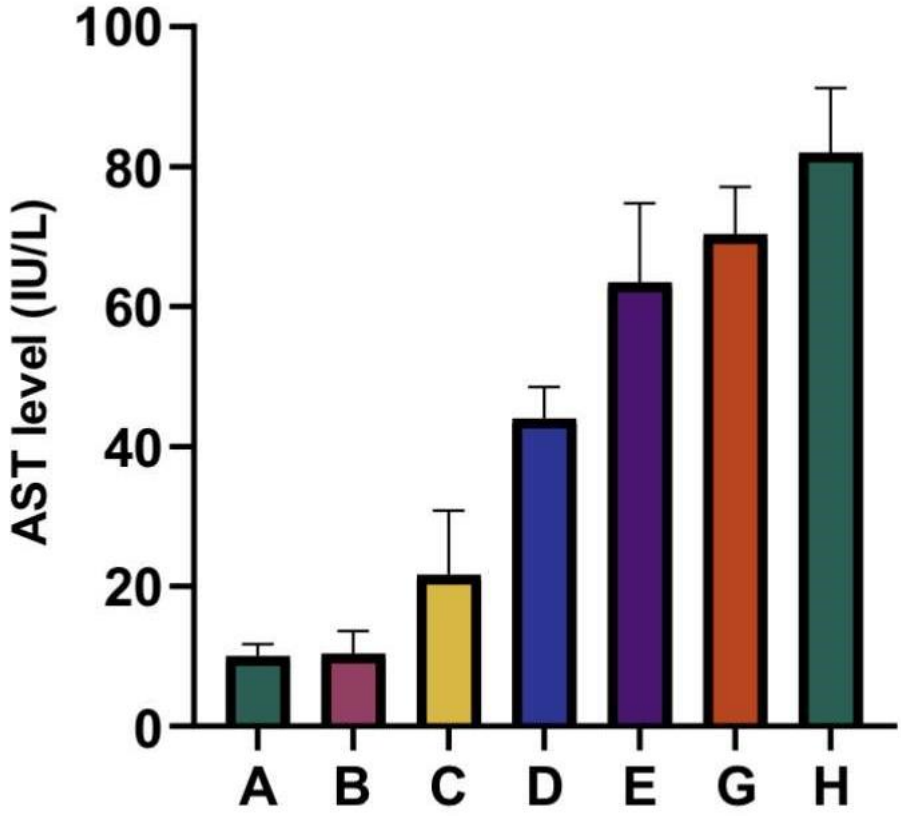


Fig 6: Effect of ethanolic leaf extract of *Mentha piperita* on AST level in mercury chloride exposed rats.

HISTOLOGICAL FINDINGS

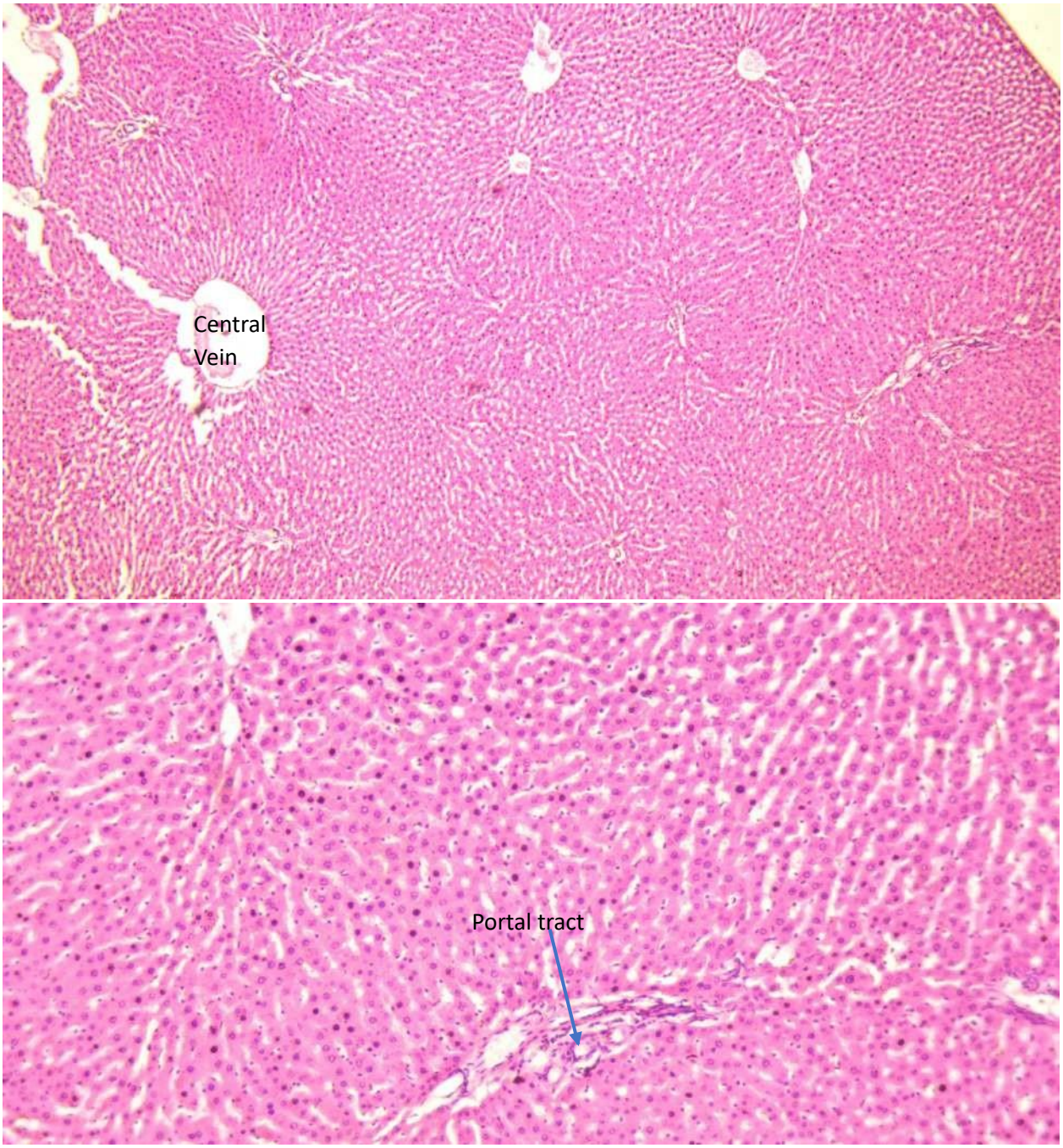


PLATE 4.1.1

D1 LIVER (800mg/kg of ethanolic leaf extract of *mentha piperita)* Section shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick. H&E:

A=X40; B=X100

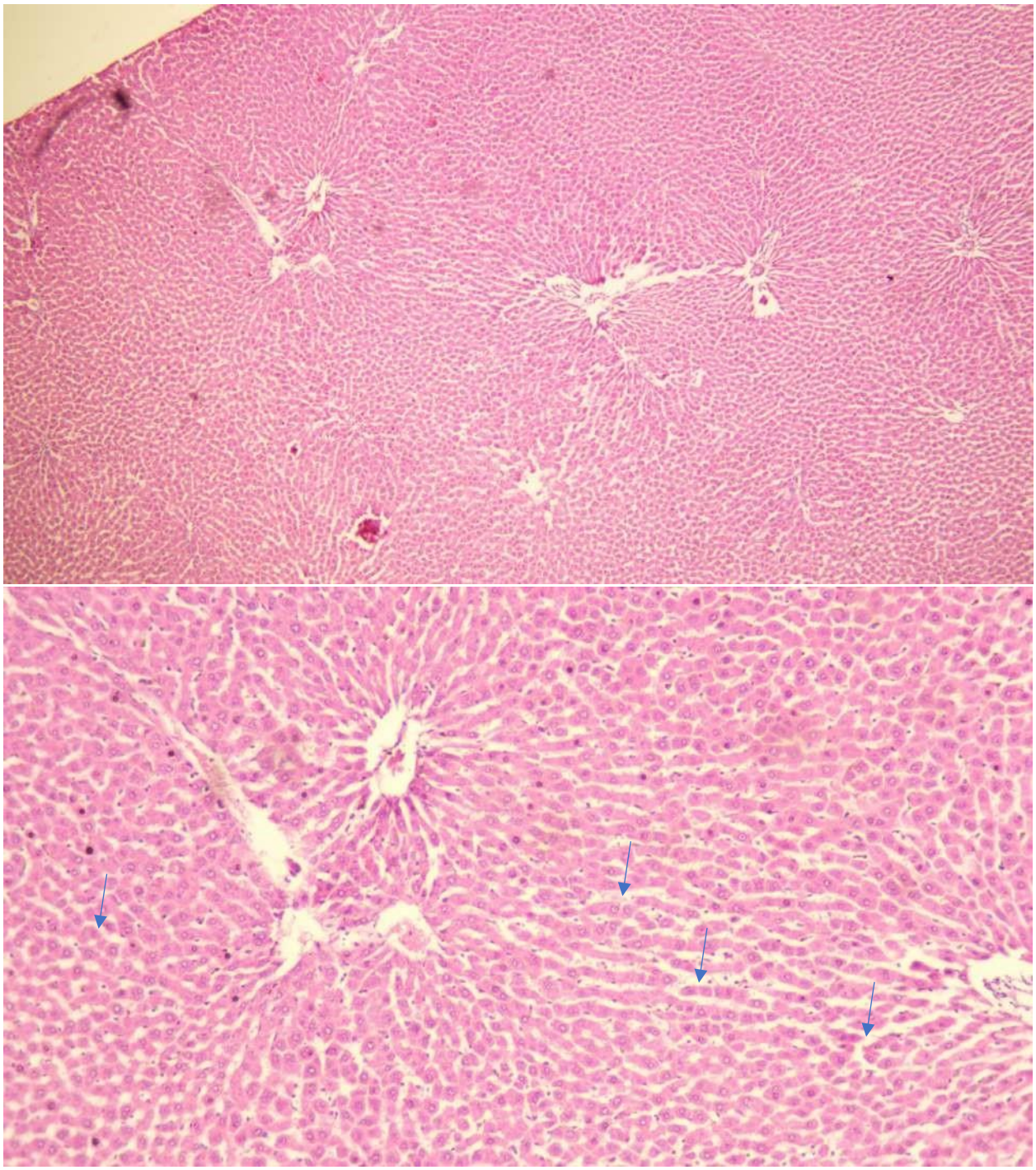


PLATE 4.1.2

D2 LIVER (800mg/kg of ethanolic leaf extract of *mentha piperita)* Section shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick. The plates are separated by sinusoids (arrows). H&E: A=X40; B=X100

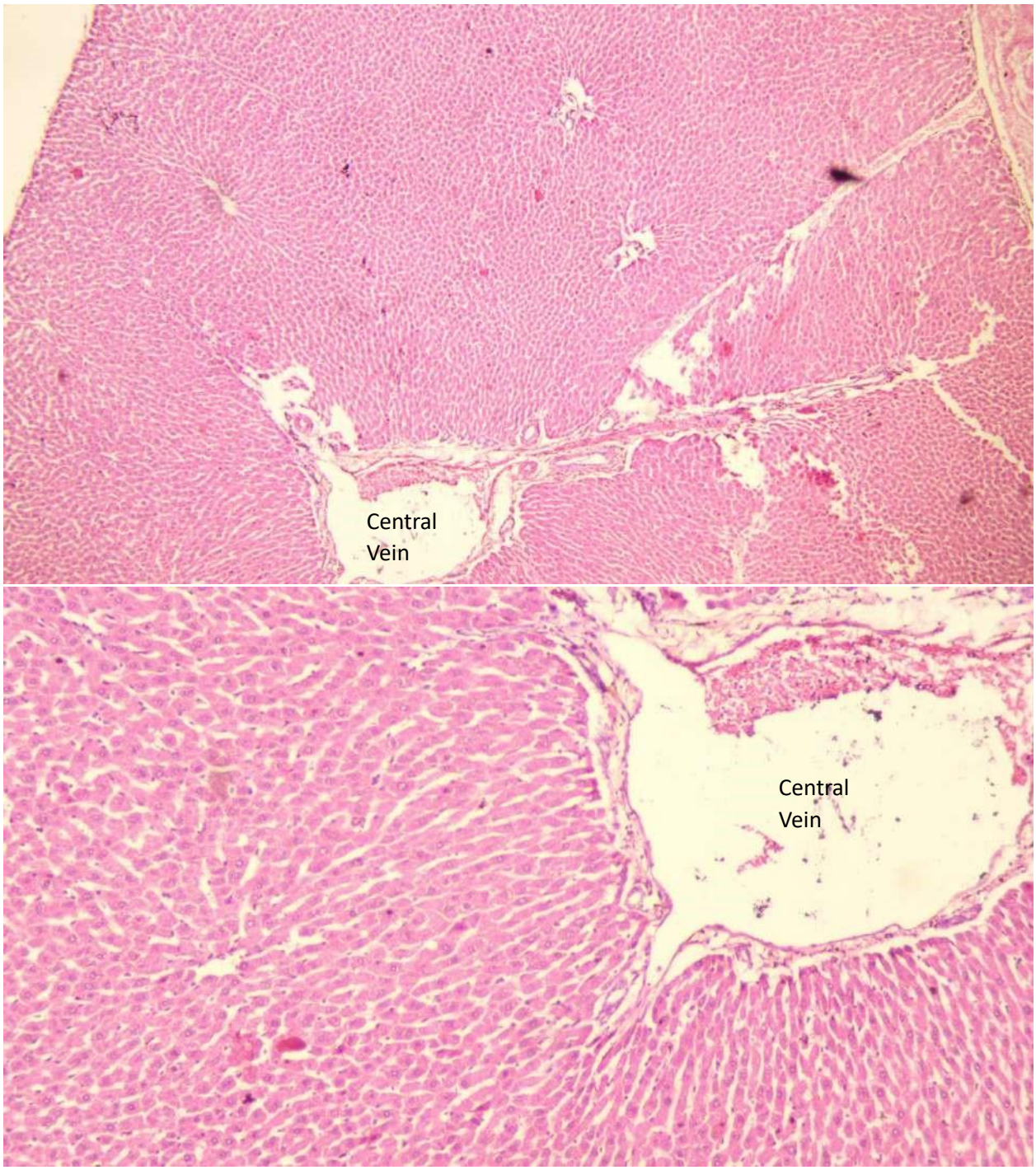


PLATE 4.1.3
D3 LIVER (800mg/kg of ethanolic leaf extract of *mentha piperita)*
H&E: A=X40; B=X100

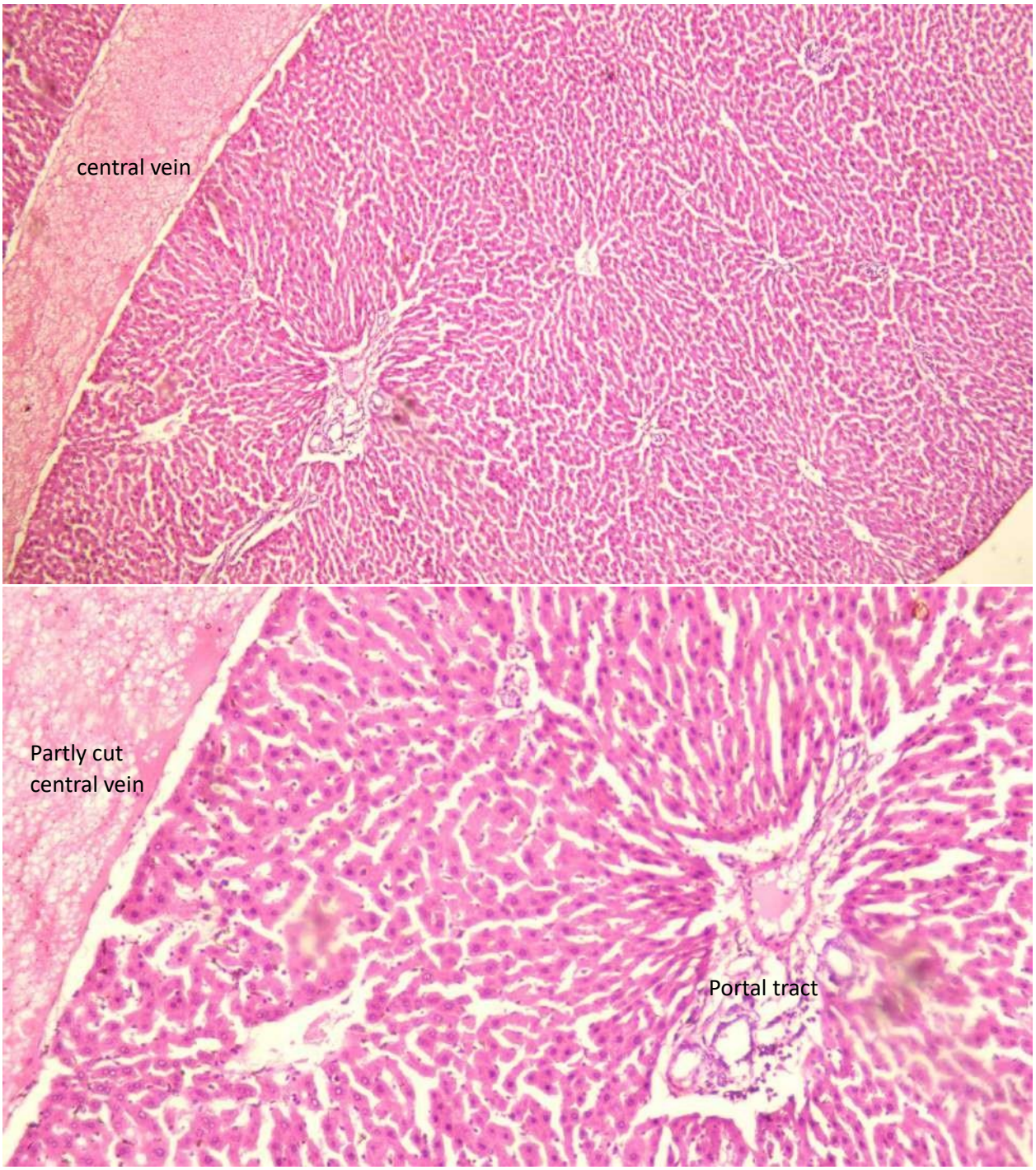


PLATE 4.2.1
E1 LIVER (1600mg/kg of ethanolic leaf extract of *mentha piperita)*
H&E: A=X40; B=X100

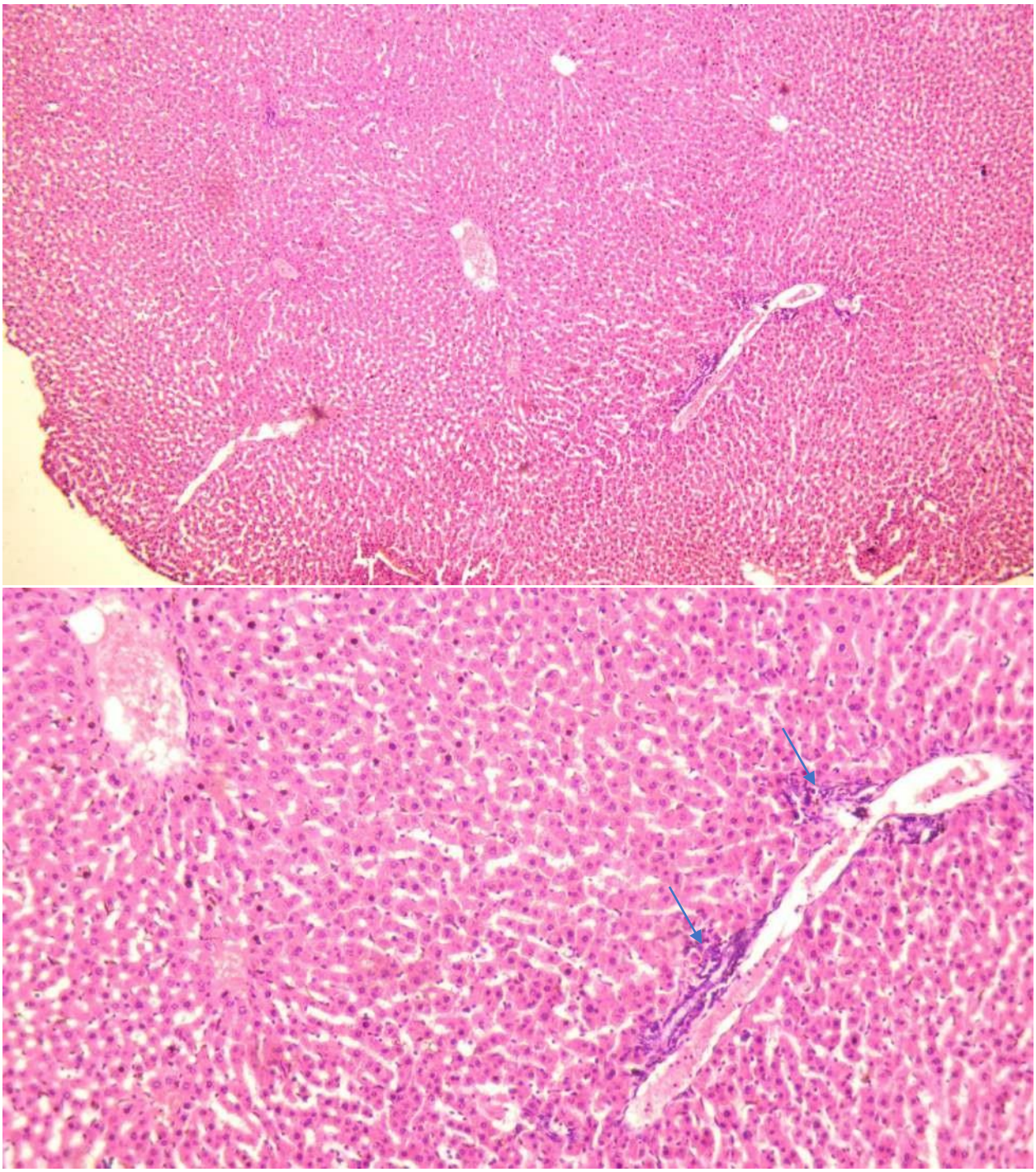


PLATE 4.2.2
E2 LIVER (1600mg/kg of ethanolic leaf extract of *mentha piperita)* with minimal portal inflammation (arrows). H&E: A=X40; B=X100

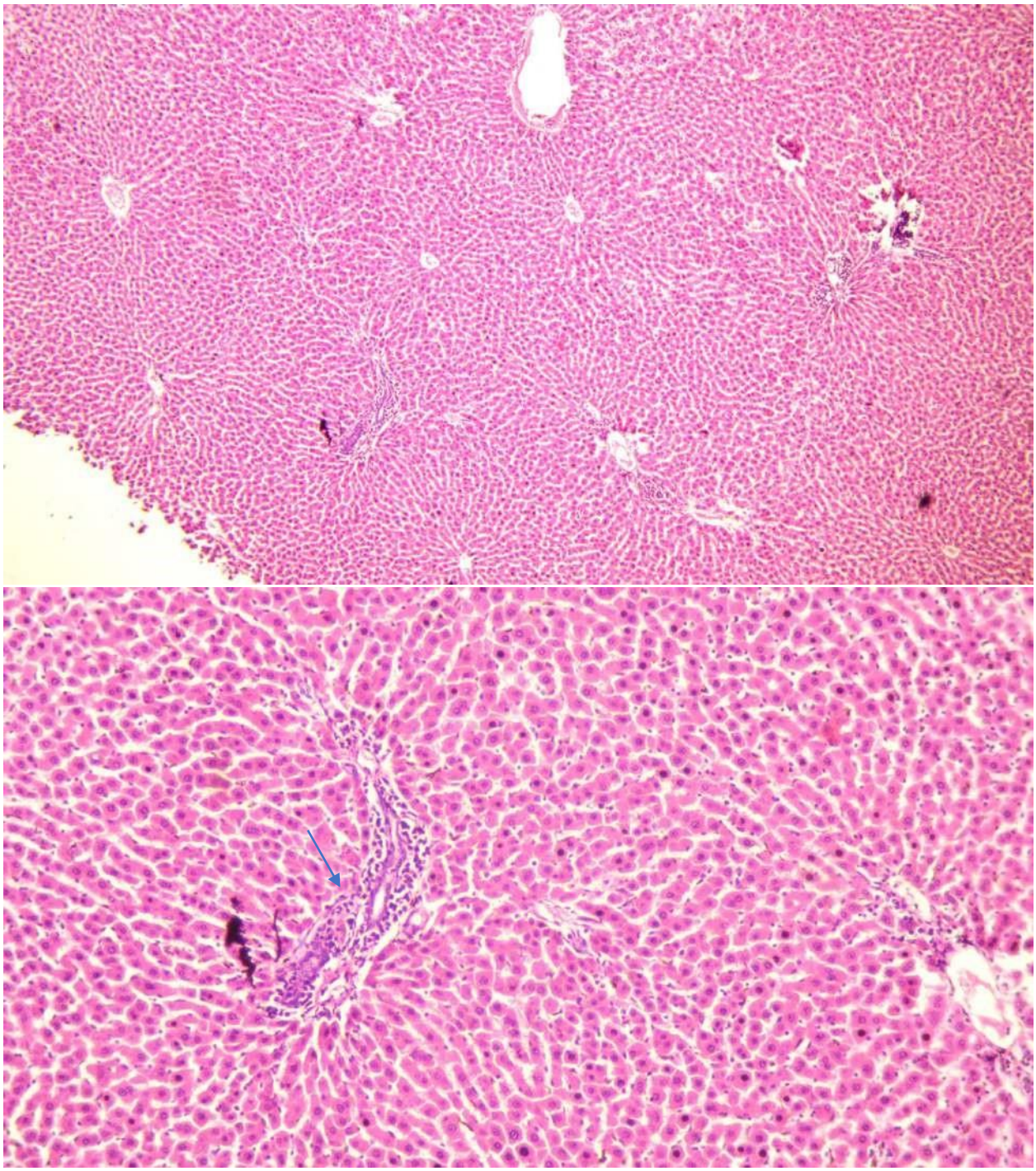


PLATE 4.2.3

E3 LIVER (1600mg/kg of ethanolic leaf extract of *mentha piperita)* Section shows normal liver with some portal inflammation (arrow). H&E: A=X40; B=X100

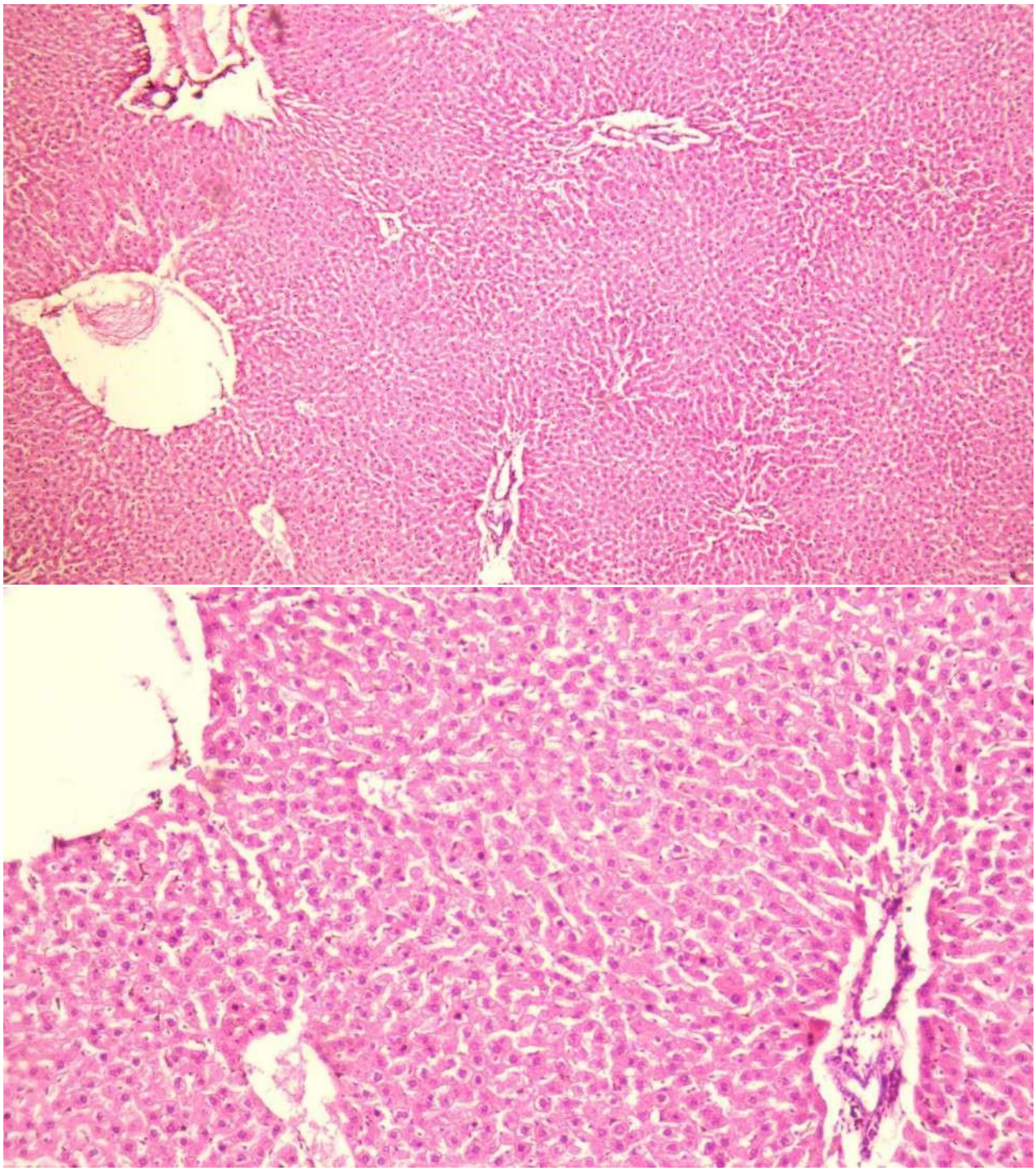


PLATE 4.3.1

G1b LIVER (800mg/kg of ethanolic leaf extract of *Mentha piperita* and 0.5mg/kg of Mercury chloride) Section shows liver tissue with some small vacuolar changes of the hepatocytes
(microsteatosis) (arrows). H&E: A=X40; B=X100

Comment: This may be a pointer to acute injury to the hepatocytes, usually by toxins

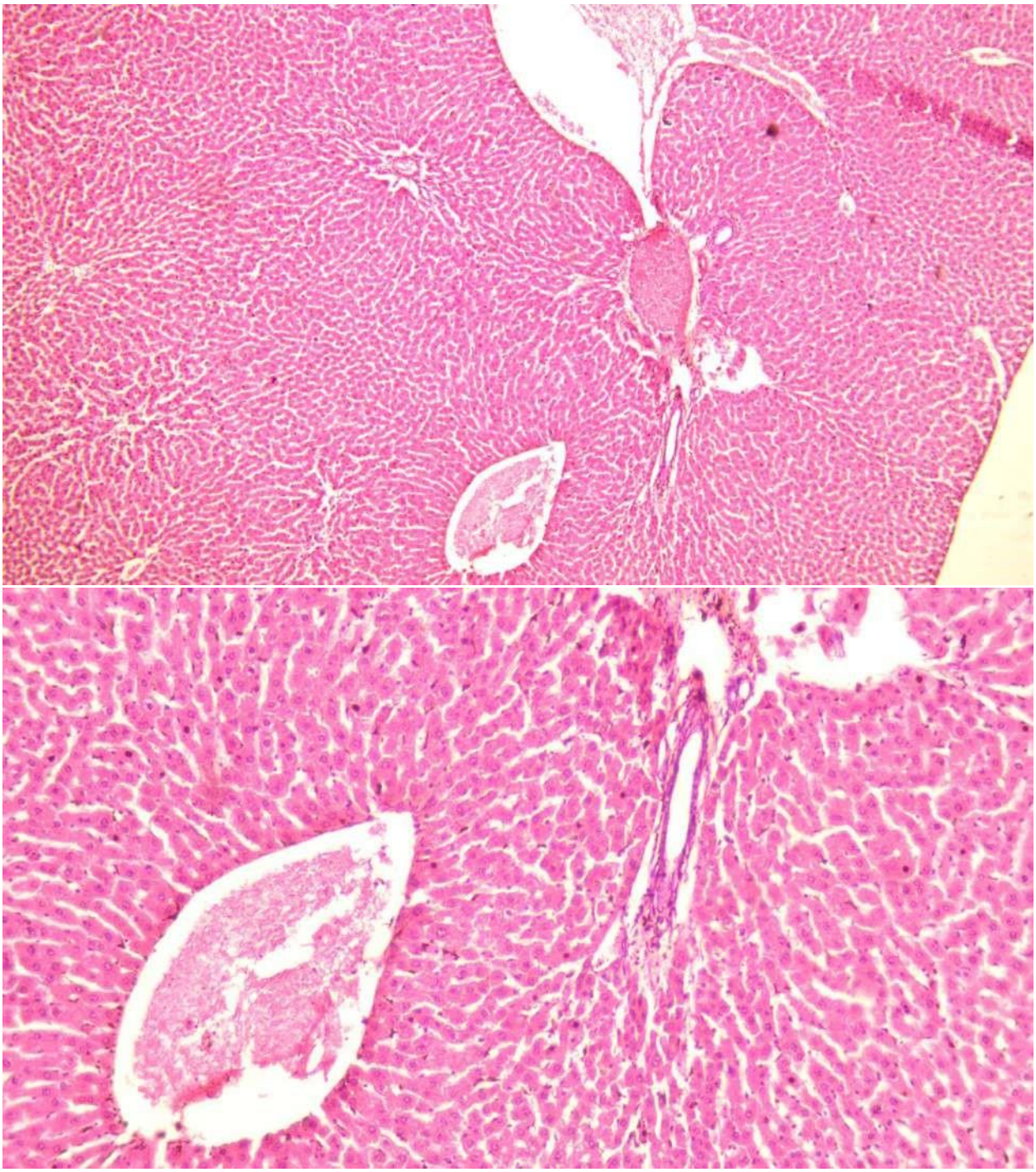


PLATE 4.3.2
G1 LIVER (800mg/kg of ethanolic leaf extract of *Mentha piperita* and 0.5mg/kg of Mercury chloride) Section shows normal liver tissue. H&E: A=X40; B=X100

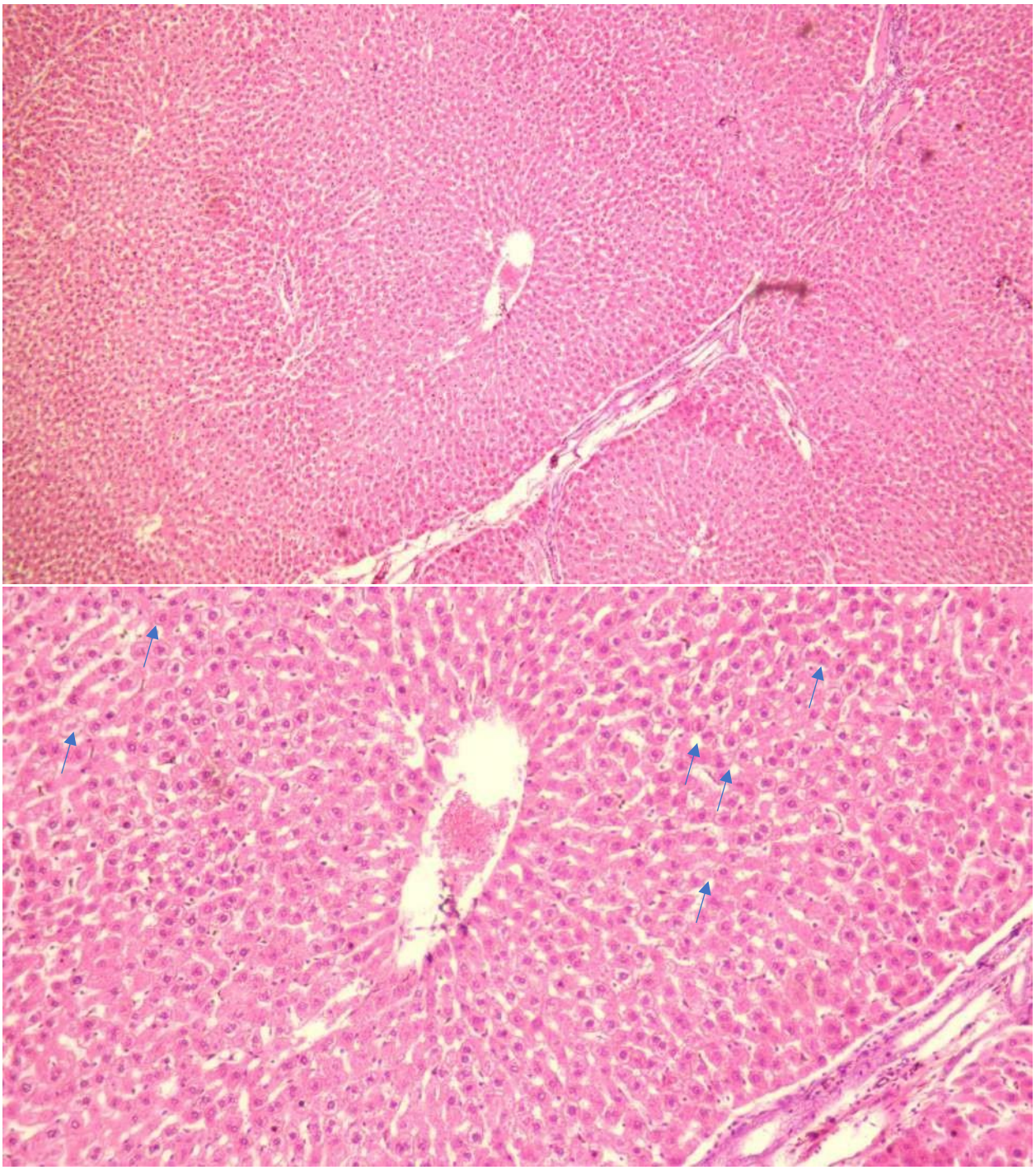


PLATE 4.3.3
G2 LIVER (800mg/kg of ethanolic leaf extract of *Mentha piperita* and 0.5mg/kg of Mercury chloride) Sections show preserved liver architecture. There is diffuse moderate steatosis involving the hepatocytes (see arrows pointing to the intracellular vacuoles). H&E: A=X40;
B=X100

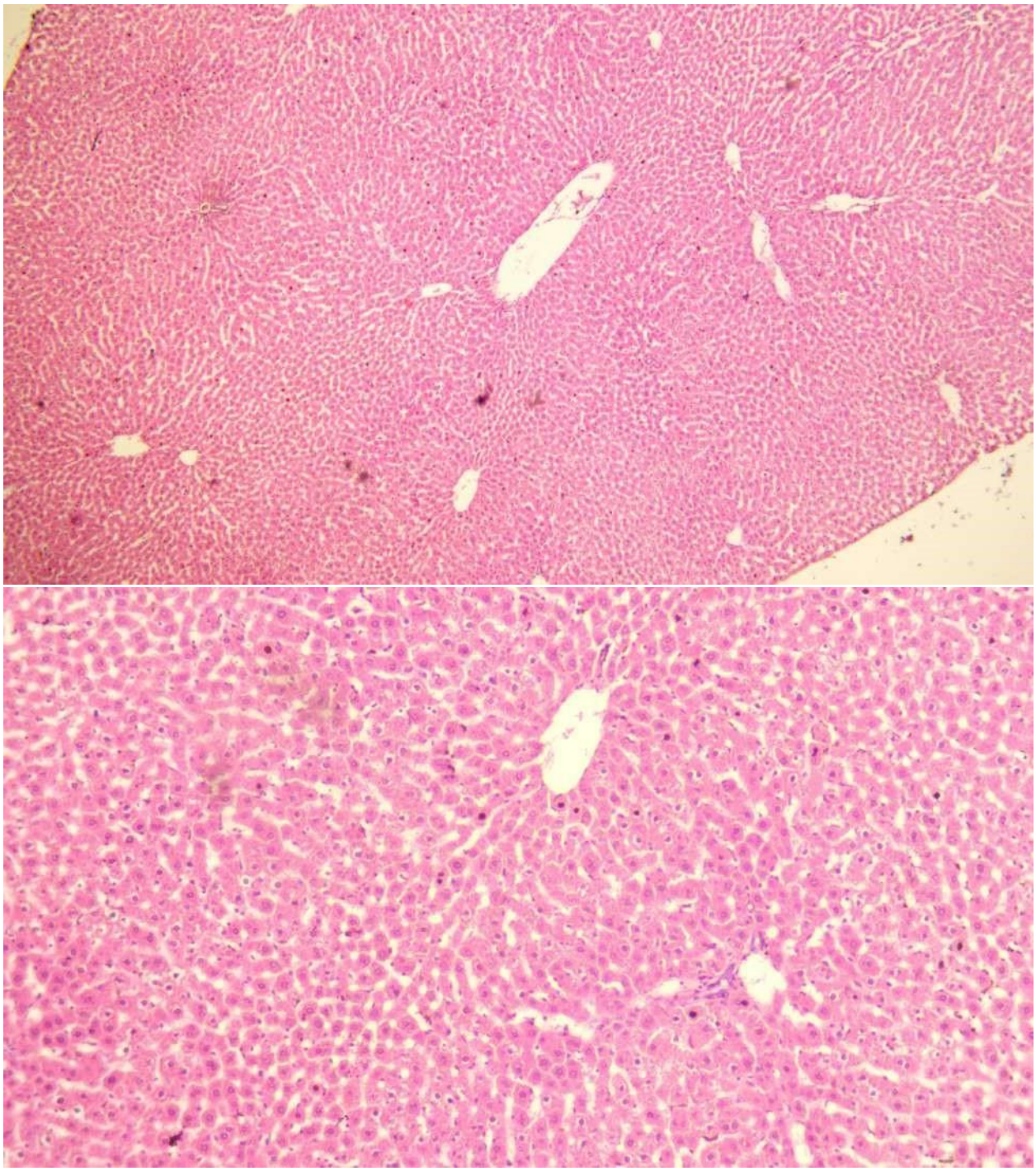
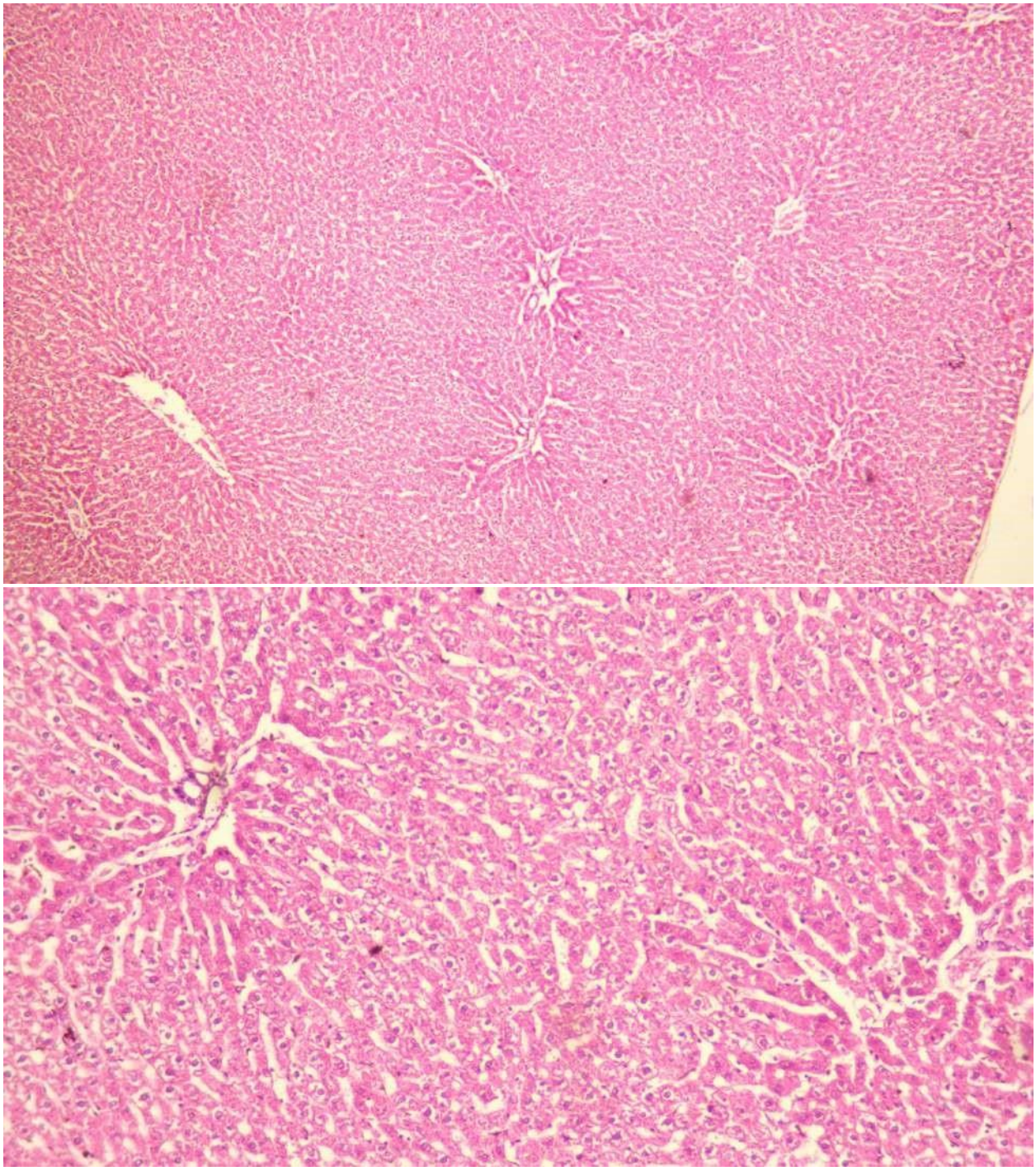


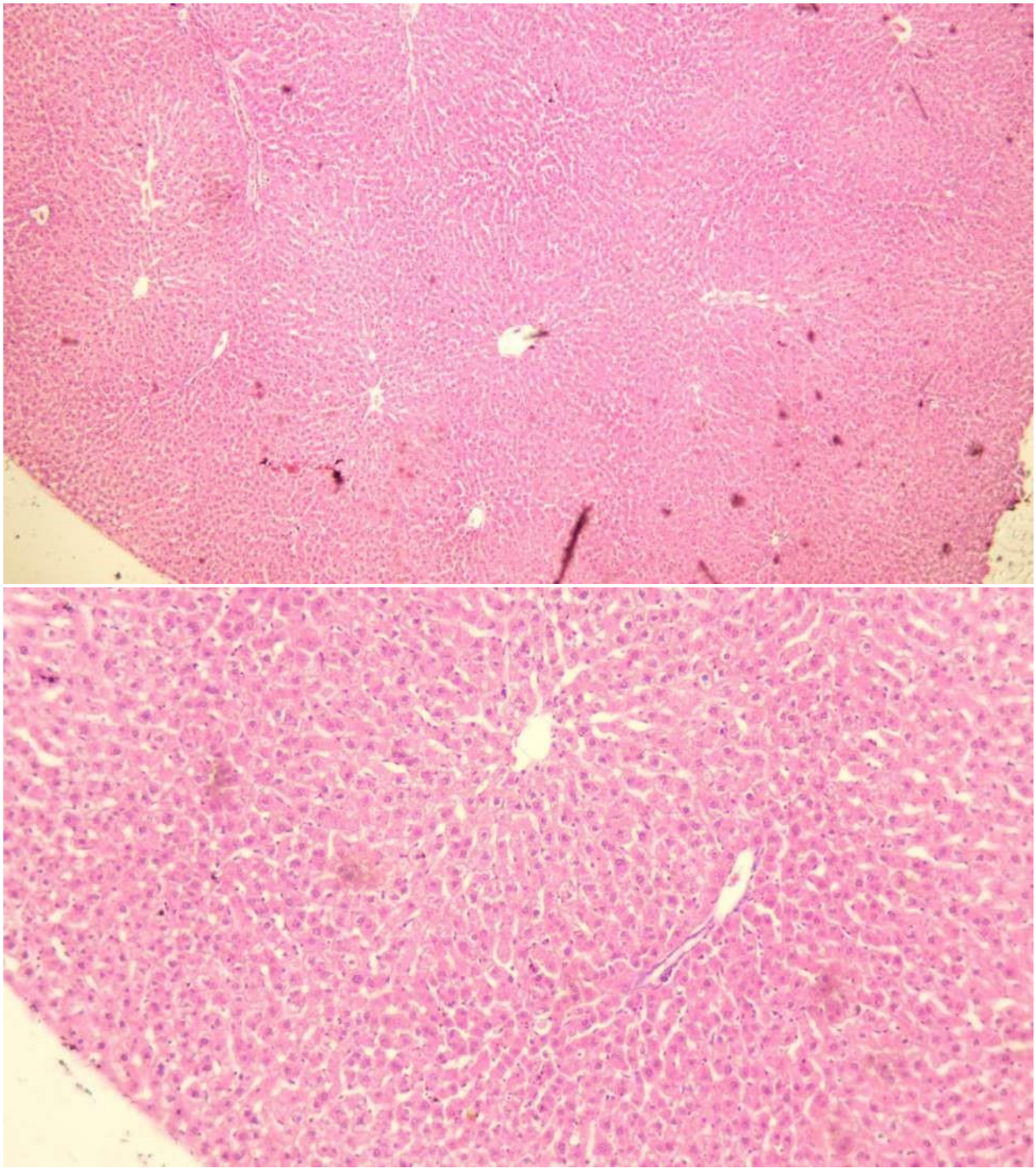
PLATE 4.3.4
G3 LIVER (800mg/kg of ethanolic leaf extract of
Mercury chloride) Sections show preserved liver architecture. There is steatosis involving the hepatocytes. H&E: A=X40; B=X100



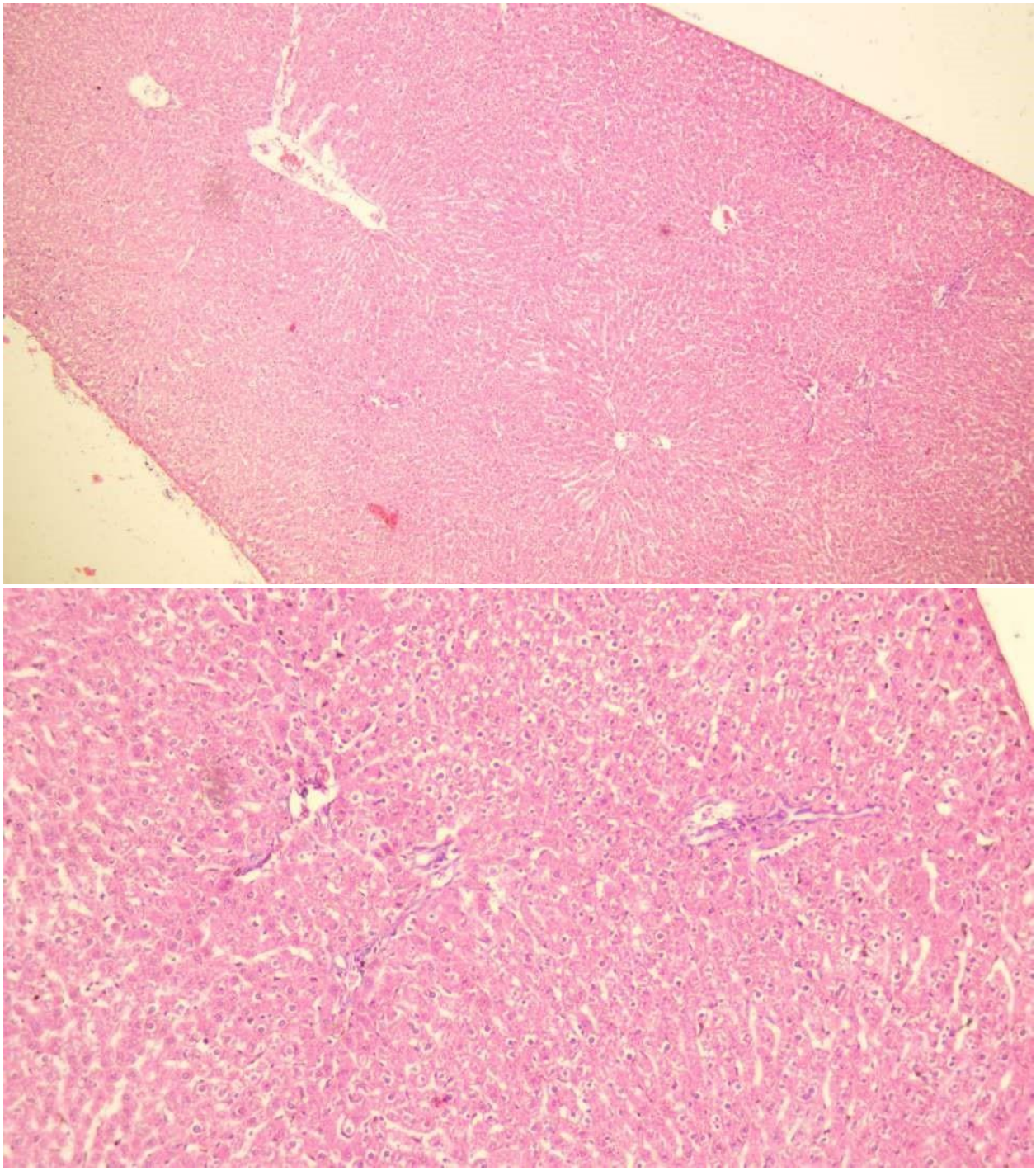
Mercury chloride) Sections show liver tissue with marked steatosis involving the hepatocytes. No inflammation is seen. H&E: A=X40; B=X100

PLATE 4.4.1

H1 LIVER (1600mg/kg of ethanolic leaf extract of Mentha piperita and 0.5mg/kg of



Mercury chloride) Sections show liver tissue with focal steatosis (circled area showing hepatocytes with vacuolar changes). H&E: A=X40; B=X100



Mercury chloride) Sections show liver tissue with marked steatosis involving the hepatocytes. No inflammation is seen. H&E: A=X40; B=X100

DISCUSSION

The present study evaluated the hepatoprotective effects of ethanolic leaf extract of *Mentha piperita* (EMP) on mercury chloride ($HgCl_2$)-induced liver toxicity in adult rats, using changes in body weight, liver weight, and liver function indices as markers of toxicity and protection.

Mercury, a known environmental toxicant, has been extensively reported to cause oxidative damage to various organs, including the liver, through mechanisms involving reactive oxygen species (ROS) generation, mitochondrial dysfunction, and lipid peroxidation (Asagba, 2010; Rojas-Lemus et al., 2019). Consistent with this, the present study found alterations in body weight and liver weight following $HgCl_2$ exposure. Rats in group A (5 mg/kg $HgCl_2$) and group B (0.5 mg/kg $HgCl_2$) showed modest increases in body weight, but these changes were not statistically significant. However, group B exhibited a relative reduction in mean liver weight, suggesting potential hepatocellular shrinkage or atrophy in response to mercuryinduced injury.

Interestingly, the control group (Group C) showed a significant increase in body weight ($p = 0.024$), indicating normal physiological growth, while all treatment groups involving EMP (Groups D–H) demonstrated trends of body weight gain but lacked statistical significance. This observation implies that EMP administration may not impede physiological weight gain and might even support it, though not strongly enough to reach significance.

In terms of relative liver weight, the highest value was observed in group A (4.58 ± 0.31), which received the highest dose of $HgCl_2$. This suggests possible liver hypertrophy or edema due to toxic insult, in agreement with earlier findings by Lund et al. (2010) that mercury compounds can cause liver enlargement through inflammatory and oxidative stress mechanisms. Conversely, groups D and E, which received 800 and 1600 mg/kg of EMP respectively, had lower relative liver weights (2.83 ± 0.49 and 3.28 ± 0.69), implying that EMP alone does not induce hepatic hypertrophy and may have protective modulation on hepatic mass.

Moreover, co-administration of EMP and $HgCl_2$ (Groups G and H) led to intermediate liver weights (4.13 ± 0.28 and 3.35 ± 0.27, respectively), suggesting that EMP, particularly at higher doses, may attenuate $HgCl_2$-induced hepatomegaly. Although these differences were not statistically significant, the downward trend aligns with previous reports that *Mentha piperita* contains phytoconstituents such as flavonoids and menthol, which have potent antioxidant and anti-inflammatory properties (Singh et al., 2015; Gharib et al., 2009). These compounds likely scavenge free radicals and stabilize hepatocyte membranes, thereby reducing oxidative burden.

While EMP alone (Groups D and E) did not significantly influence body weight or liver weight compared to controls, their co-administration with $HgCl_2$ showed partial reversal of mercury's effects. This supports the hepatoprotective role of EMP and aligns with similar findings in the literature where *Mentha piperita* mitigated chemically induced hepatotoxicity (Alqasoumi et al., 2012). Although the protective trends observed were not statistically significant in most cases, the results are biologically relevant and suggest that EMP may provide some degree of protection against mercury-induced hepatic alterations, especially at higher doses.

In this study, rats exposed to $HgCl_2$ alone (Groups A and B) demonstrated moderate increases in body weight and elevated liver enzyme levels, particularly ALP. Group A (5 mg/kg $HgCl_2$) had a notably high ALP level (52.33 ± 11.09 IU/L), reflecting hepatic membrane damage and possible biliary obstruction, which aligns with earlier findings (Kale et al., 2020). Interestingly, the ALT levels in $HgCl_2$-exposed groups (Groups A and B) were not markedly elevated, and in fact, Group B had a slightly lower ALT (12.33 ± 2.91 IU/L) than the control group. However, AST levels were significantly reduced in Group B (10.33 ± 3.28 IU/L) compared to the control (21.66 ± 9.17 IU/L), though this difference was not statistically significant ($p = 0.294$), possibly due to early-stage liver insult or compensatory mechanisms (Rojas-Lemus et al., 2019).

Notably, EMP alone (Groups D and E) significantly reduced ALP and ALT levels compared to the mercury-treated groups. For instance, ALP was reduced to 4.42 ± 0.28 IU/L and 9.59 ± 1.89 IU/L in groups D and E, respectively. This strongly suggests a hepatoprotective effect of *Mentha piperita*, likely mediated through its antioxidant phytochemicals such as flavonoids, menthol, and rosmarinic acid, known to stabilize cell membranes and counteract oxidative stress (Gharib et al.,
2009; Singh et al., 2015). The ALT levels in these groups also showed a reduction (5.22 ± 1.25 IU/L and 6.60 ± 0.85 IU/L), with group D demonstrating statistical significance ($p = 0.026$), suggesting protection against hepatocellular damage.

Unexpectedly, the AST levels were elevated in the EMP-only groups (43.98 ± 4.51 and 63.43 ± 11.32 IU/L for groups D and E, respectively) and further increased in the EMP + $HgCl_2$ groups (70.33 ± 6.77 and 81.96 ± 9.23 IU/L for groups G and H), all showing statistical significance ($p < 0.05$). While AST is less liver-specific and can originate from muscle or heart, its elevation may reflect a non-specific tissue response or suggest that high-dose EMP may induce a mild stress response in extrahepatic tissues (Alqasoumi et al., 2012). Nevertheless, the concurrent reductions in
ALT and ALP point toward preserved hepatocyte integrity.

Furthermore, relative liver weights were highest in Group A (4.58 ± 0.31 g), indicating hypertrophy or inflammatory swelling, while EMP treatment (Groups D and E) reduced liver weights (2.83 ± 0.49 and 3.28 ± 0.69 g), reinforcing the extract's protective potential. Co-treatment groups (G and H) showed intermediate liver weights and enzyme levels, indicating partial amelioration of $HgCl_2$ toxicity.

Collectively, these findings suggest that EMP confers hepatoprotective effects, particularly through downregulation of ALP and ALT activities and attenuation of liver hypertrophy. This aligns with previous studies where *Mentha piperita* extract was shown to restore liver enzymes toward normal values in chemically induced hepatotoxic models (Alqasoumi et al., 2012; Boukhatem et al., 2014).

Histological findings shows the photomicrography of liver tissue changes that occurred in the liver tissue after administration of mercury chloride and mentha piperita. Plate 4.1.1 and 4.1.2 shows normal liver with normal portal tracts, central vein and liver plate of hepatocytes one-to-two-cell thick of group D. In Plate 4.1.2 the plates are separated by sinusoids. Plate 4.2.1 shows the histoarchitecture of a normal liver in group E. Plate 4.2.2 and 5.2.3 shows the histoarchitecture of the liver tissue with signs of minimal and some portal inflammation.Plate 4.3.1 shows the liver tissue with some small vacuolar changes of the hepatocytes (microsteatosis). This may be a pointer to acute injury to the hepatocytes, usually by toxins. Plate
4.3.3 and Plate 4.3.4 show preserved liver architecture. There is diffuse moderate steatosis involving the hepatocytes. Plate 4.4.1 show liver tissue with marked steatosis involving the hepatocytes. No inflammation is seen. Plate 4.4.2 and 4.43 shows liver tissue

with focal steatosis. The circled area in Plate 4.4.2 shows hepatocytes with vacuolar changes while in Plate 4.4.3 no inflammation is seen.

CONCLUSION

The findings suggest that ethanolic leaf extract of Mentha piperita (EMP) possesses significant hepatoprotective effects against mercury chloride-induced toxicity. EMP's ability to mitigate liver damage, reduce liver enzyme levels, and improve liver function indicates its potential as a therapeutic agent.

These results support the traditional use of Mentha piperita in folk medicine for liver-related ailments.

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