

EVALUATION OF EFFICACY OF *OSCIMUM SCANTUM* AGAINST CCl₄ INDUCED LIVER TOXICITY IN RAT

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ABSTRACT

Oscimum scantum have been reported to exhibit varying degrees of hepatoprotection against the CCl₄ induced liver dysfunction in rats. The present work was carried out to investigate the potential hepatoprotective action of *Oscimum scantum* whole plant powder against CCl₄ induced liver damaged Wister Rat Model. Blood and tissue biochemical parameters of liver were studied for evaluating the hepatoprotection action. *Oscimum scantum* whole plant powder is compared with Silymarin by standard protocol and is found to have better hepatoprotective action, thus it may act even in humans as a potent liver tonic.

KEY WORDS: *Oscimum scantum*, CCl₄, Wister Rat, liver, Silymarin, hepatoprotection.

1. INTRODUCTION

Liver disorders constitute a major health problem in India. There is scarcity of effective modern drugs for the treatment of liver disorder like Jaundice. Many herbal preparations have been marketed for the same. *Oscimum scantum* is widely used as one of the component by Folk healers for treatment of jaundice. The current investigation on *Oscimum scantum* as to the hepatoprotective activity was undertaken as an extension of my earlier work on *Argemone mexicana* and *centella-asiatica*.

Literature pertaining to *Oscimum scantum* suggests that Tulsi has been used for thousands of years as a prime herb in Ayurvedic treatment, for its diverse healing properties. Tulsi is considered to be an adaptogen, balancing different processes in the body and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity.

Tulsi's extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Traditionally, tulsi is taken in many forms: as an herbal tea, dried powder, fresh leaf, or mixed with ghee. Essential oil extracted from Karpoora Tulsi is mostly used for medicinal purposes and in herbal cosmetics. Widely used in skin preparations for its anti-

bacterial activity. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects.

Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its significant amount of eugenol (1-hydroxy-2-methoxy-4-allylbenzene). Studies have also shown Tulsi to be effective for diabetes, by reducing blood glucose levels. The same study showed significant reduction in total cholesterol levels with Tulsi. Another study showed that Tulsi's beneficial effect on blood glucose levels is due to its antioxidant properties. Tulsi also shows some promise for protection from radiation poisoning and cataracts.

2. MATERIALS AND METHODS

Oscimum scantum plants were collected from Rajgurunagar and Narayangaon (Pune), India. Herbaria of the plant were authenticated by BSI (Botanical Survey of India), Pune, India. After collection of the required quantity, it was carefully segregated, washed and dried in shade to constant weight. The plant material was kept in preset oven for eight days at 45°C. The dried plant free of moisture was powdered in high speed electronic mixer and sieved through a BSS Mesh No. 85 sieve and stored in an airtight container.

Sixty Wistar rats of both sexes (30 male and 30 female) were procured from Raj Biotech (INDIA) Ltd., Pune, India. The animals were housed in standard rat cages and were fed with commercially available rat feed pellets supplied by AMRUT feeds. The animals were given measured volume (250 ml) of drinking water and weighed amount (200 g) of food during the experiment. All animals used for the study were in the weight range of 100 - 130g. The animals were randomly divided into five groups of twelve (6 male and 6 female)

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animals each. The male and female rats were housed in separate cages.

After an acclimatization period of fourteen days the rat cages were randomly assigned the following treatments; Group I – Normal (Vehicle) control, Group II - toxicant (CCl₄) control, Group III - toxicant (CCl₄) recovery, Group IV - plant (*Oscimum scantum*) control, Group V - CCl₄ + Silymarin treated. The animals from Group I received an intra peritoneal (i.p.) injection of 0.5ml of liquid paraffin and those from Group II, III, IV and V received an i.p. injection of 0.7ml/Kg of CCl₄ (Pandey and Chaturvedi 1969; Zimmerman, 1978; Sane, 1995; Meghana, 1999) in 0.5ml liquid paraffin per animal on the first day of the study. The animals from Group I, II and III received an oral dose of 2ml of distilled water (D/W) once daily. The animals from Group IV received an oral dose of 0.50g/Kg of sieved whole plant powder of *Oscimum scantum* suspended in 2ml of distilled water per animal. The animals from Group V received an oral dose of 0.007g/Kg (Silymarin Clinical Update, 1995) of Silymarin suspended in 2ml of distilled water per animal. The animals from Group I, II, IV and V were sacrificed on the fourth day (72 hours after dosing) and those from Group III were sacrificed on seventh day of the study. The chart showing daily dose regime is given below in Table No. I.

Table-1: DAILY DOSE REGIME

DAYS	Group I Vehicle Control	Group II CCl ₄ control	Group III CCl ₄ treated natural recovery	Group IV CCl ₄ + plant slurry treated	Group V Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. & 0.5gm/kg plant slurry in 2cc d/w oral	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.5gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.5gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

- Note:**
1. The above dosage is for an individual animal of the group.
 2. The number of animals in each group = 6.
 3. i.p. = intra peritoneal.
 4. d/w = distilled water
 5. liq. paraffin = liquid paraffin.

Before sacrificing the animals were weighed. The food supply was stopped twelve hours before sacrifice. All animals were weighed before sacrifice. Blood was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood biochemical assays of some selected parameters like Alkaline phosphatase (AlkP), Glucose, Cholesterol, Triglycerides (TG), Aspartate transferase (AST) and Alanine Transferase (ALT) was carried out as per the standard kits (Raichem, division of Hemagen diagnostic, inc. San Diego, CA 92111-1203). After sacrifice, liver was excised, rinsed in saline, blotted and weighed. Liver to body weight ratio was calculated of all animals. A small 5 mm piece of liver from the largest lobe was cut and fixed in Bouin's fixative for 24 hours. The tissues after fixations were processed, cut into sections of 1 µm and 7 µm and stained. The stained slides were observed, photographed and preserved. The remaining liver was used for the estimation of tissue biochemical assays, which included DNA, RNA, Alkaline phosphatase, Glycogen and Cholesterol. All the assays were done using standard Diagnostic kits, DNA was estimated by the method of Munro (1967) and Burton (1956). While RNA was estimated by the method of Munro (1967) and Ceriotti (1955).

3.RESULTS AND DISCUSSION

Biochemical Parameters

The use of CCl₄ as a hepatotoxicant in animal models is well documented. Hepatic damage caused by CCl₄ is very specific except at higher doses and is reversible to low doses. The biochemical and histopathological changes in liver exposed to CCl₄ are well documented (Rouiller, 1964; Zimmerman, 1978; Timbell, 1982; Poole, 1989; and Hayes, 1989). It has a destructive effect on the membranes of the hepatocytes and interferes with cellular metabolism and transport. A single dose leads to centrilobular necrosis and fatty liver. Within few minutes, there is injury to endoplasmic reticulum leading to functional defects of the hepatocyte. Multiple biochemical manifestations of hepatic injury can be recorded. Irrespective of the route of administration, CCl₄ leads to centrilobular necrosis and steatosis. Biochemical changes in the blood adequately reflect status of the injury. Serum enzymes levels increase with cytoplasmic enzymes, reaching their peak level within 12 hours of exposure (Rouiller, 1964; Zimmerman, 1978). Mitochondrial enzymes reach their

peak level within 36 hours (Zimmerman,1978; Rouiller,1964). Enzymes common to both mitochondria and cytoplasm reach their peak level around 24 hours (Zimmerman,1978; Rouiller,1964). The process of recovery begins within 24 to 48 hours (Zimmerman,1978; Rouiller,1964) after exposure of CCl_4 .

CCl_4 causes hepatic damage through the production of trichloromethyl (CCl_3) free radical. The metabolism of CCl_4 releases CCl_3 free radical, which initiates peroxidation and cleavage of fatty acids in the membranes (Rouiller,1964; Pandey and Chaturvedi, 1969; Zimmerman,1978; Schever,1988; Poole and Leslie,1989; Wallace Hayes, 1989; Sane, 1995; Meghana, 1999). Observations published earlier indicate that CCl_4 causes accumulation of fat in the liver especially by interfering with the transfer of triglycerides from the liver into the plasma (Schever,1988). Many clinical conditions that cause an increase in cholesterol levels also cause increase in triglycerides (TG). Blockage of the secretion of hepatic TG into the plasma is the basic mechanism underlying the fatty liver induced in the rat by CCl_4 . This causes elevated amounts of fats predominantly TG in the parenchymal cells (Zimmerman,1978; Schever,1988).

Aspartate and Alanine aminotransferase are present in high concentration in liver. Due to necrosis of hepatocyte or abnormal membrane permeability, these enzymes are released from the hepatocytes into the blood and their levels in the blood increase (Rouiller,1964; Zimmerman,1978; Poole,1989; and Hayes,1989). Alanine aminotransferase (ALT) is a sensitive indicator of acute liver damage and elevation of this enzyme in non-hepatic disease is unusual. ALT is more selectively a liver parenchymal cell enzyme than is aspartate aminotransferase (AST). The transaminases being more stable than other enzymes under the laboratory conditions are better indices for evaluating the extent of hepatic damage. Alkaline phosphatase is a membrane bound enzyme and its elevation in plasma indicates membrane disruption in the organ (Rouiller,1964;Zimmerman,1978; Poole,1989; and Hayes,1989). Alkaline phosphatase, although is not a liver specific enzyme, but liver is the major source of alkaline phosphatase. The level of this enzyme increases in cholestasis. Levels of Lactic dehydrogenase reach a peak at 12 hours after CCl_4 treatment.

CCl_4 treatment causes significant increase in blood and tissue biochemical parameters which were studied. Reduction in RNA values indicates the toxicant induced changes in protein synthesis. A change in DNA levels of liver observed in the present study indicates CCl_4 induced cellular hepatic damage.

Significant variations in tissue glycogen and plasma glucose levels after treatment with CCl_4 indicate impairment of liver metabolism. CCl_4 treatment causes a drop in tissue glycogen levels. The plant treated group shows a further decrease in the tissue glycogen as compared to CCl_4 control group. From the results of the blood parameters, it is evident that CCl_4 causes hyperglycemia in animals of CCl_4 control group. Treatment of *Oscimum scantum* whole plant powder lowers the blood glucose level bringing it close to normal values. Values of blood glucose in recovery group animals are close to control group values.

Treatment of animals having CCl_4 induced hepatic injury with *Oscimum scantum* causes an increase in tissue alkaline phosphatase and cholesterol levels and blood alkaline phosphatase, TG, AST and ALT levels as compared to those of CCl_4 control group animals. Blood cholesterol levels of *Oscimum scantum* plant treated group animals showed a significant reduction as compared to CCl_4 control. This reduction in cholesterol levels was lower than those in animals of natural recovery group.

The Group IV animals treated with only the *Oscimum scantum* plant slurry show significantly comparable levels of blood alkaline phosphatase, cholesterol, TG, AST and ALT levels as compared to control group animals.

Liver - Body Weight Changes: Liver to body weight ratio of the animals from all the groups showed no significant changes in their values. This observation can be attributed to the lower dose of CCl_4 used for the study.

General Observations

Animals from all groups showed no abnormal behaviour in food and water consumption. The food consumptions of animals from CCl_4 control, CCl_4 + *Oscimum scantum* plant slurry treated and CCl_4 + Silymarin group decreased significantly. The CCl_4 recovery group animals showed significant decrease up to the fourth day of the treatment and then they showed an increase. This indicates that the animals are

recovering from the toxicity induced by the CCl_4 similar observations were noted with the trends in water consumption by treated animals.

TABLE - II:
BLOOD AND TISSUE BIOCHEMICAL PARAMETERS

PARAMETER (T) TISSUE (B) BLOOD	Group I Vehicle Control	Group II CCl_4 control	Group III CCl_4 treated natural recovery	Group IV CCl_4 + plant slurry treated	Group V Silymarin treated
BLOOD BIOCHEMICAL PARAMETERS					
Glucose (B)	76.50	150.40	65.40	79.50	68.70
Alk PO_4 (B)	98.50	115.00	118.40	112.40	110.40
Cholesterol(B)	40.50	63.50	56.40	62.00	48.60
Triglycerides(B)	56.50	89.40	63.50	87.80	61.00
AST(B)	30.00	38.00	34.80	34.50	3.50
ALT(B)	26.00	39.50	35.10	43.00	34.00
TISSUE BIOCHEMICAL PARAMETERS					
DNA(T)	8.50	8.40	14.00	11.20	6.50
Alk PO_4 (T)	57.50	51.20	27.00	51.80	38.00
RNA(T)	140.00	120.80	58.80	78.20	66.00
Glycogen(T)	5.10	4.60	3.80	4.70	3.80
Cholesterol(T)	45.60	68.20	36.30	44.20	38.40
LIVER TO BODY WEIGHT RATIO					
LIVER TO B/W RATIO	0.031	0.031	0.031	0.031	0.030

Liver Histology: The light microscopy of normal rat liver reveals almost regular structures. The hepatocytes in thin sections appear to radiate from the central vein. The hepatocytes are polygonal with well-defined borders, with single nucleus in each. The thin sections show a portal tract with distinct endothelial lining surrounded by terminal portal venules, hepatic artery and small bile duct. (Plate LM 01). (Rouiller, 1964; Zimmerman, 1978; Timbell, 1982; Poole, 1989; Hayes, 1989; Bloom, 1994).

The rat liver after CCl_4 treatment (3 days) shows distinct centrilobular necrosis with hepatocytes of these areas showing distinct vacuolation. The nucleus appears pyknotic in these cells. The periportal region appears normal. There is distention of sinusoidal lumen in the centrilobular area. There is also distinct enlargement of hepatocytes and few areas show infiltration of mononuclear cells especially near the portal veins. (Plate LM 02). (Rouiller, 1964; Zimmerman, 1978; Timbell, 1982; Poole, 1989; Hayes, 1989; Bloom, 1994).

The rat liver after CCl_4 treatment (7 days) shows there is distinct absence of lipid accumulation and reduced mitochondrial activity as compared to CCl_4 treated (3 days) cells. The microvilli appear normal. There is however, abundance of rER in hepatocytes. (Plate LM 03).

The liver of the rats after combined treatment of CCl_4 and *Oscimum scantum* (Plate LM 04) shows mild congestion in some of the sinusoids. The dilation of sinusoids is evident in the centrilobular areas. The

vacuolation seen after CCl_4 treatment is significantly absent.

The liver of the rats after combined treatment of CCl_4 and Silymarin shows more focused regions of recovery. The dilation of sinusoids is evident in the centrilobular areas. The vacuolation seen after CCl_4 treatment is significantly absent (Plate LM 05).

Plate LM1: Low power light micrograph of normal rat liver showing a large portal vessel

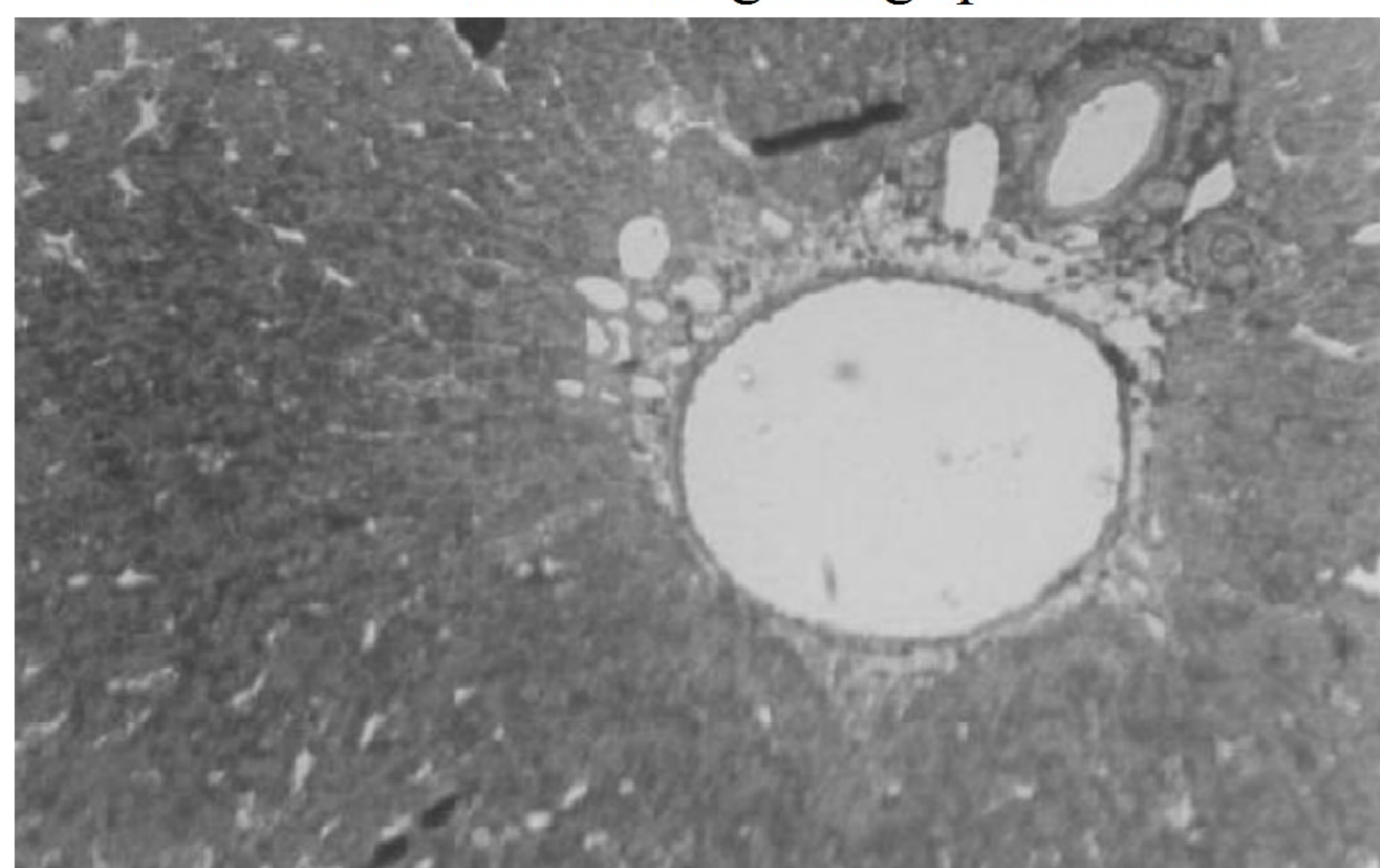


Plate LM 2: Low power light micrograph of rat liver after CCl_4 treatment showing necrosis

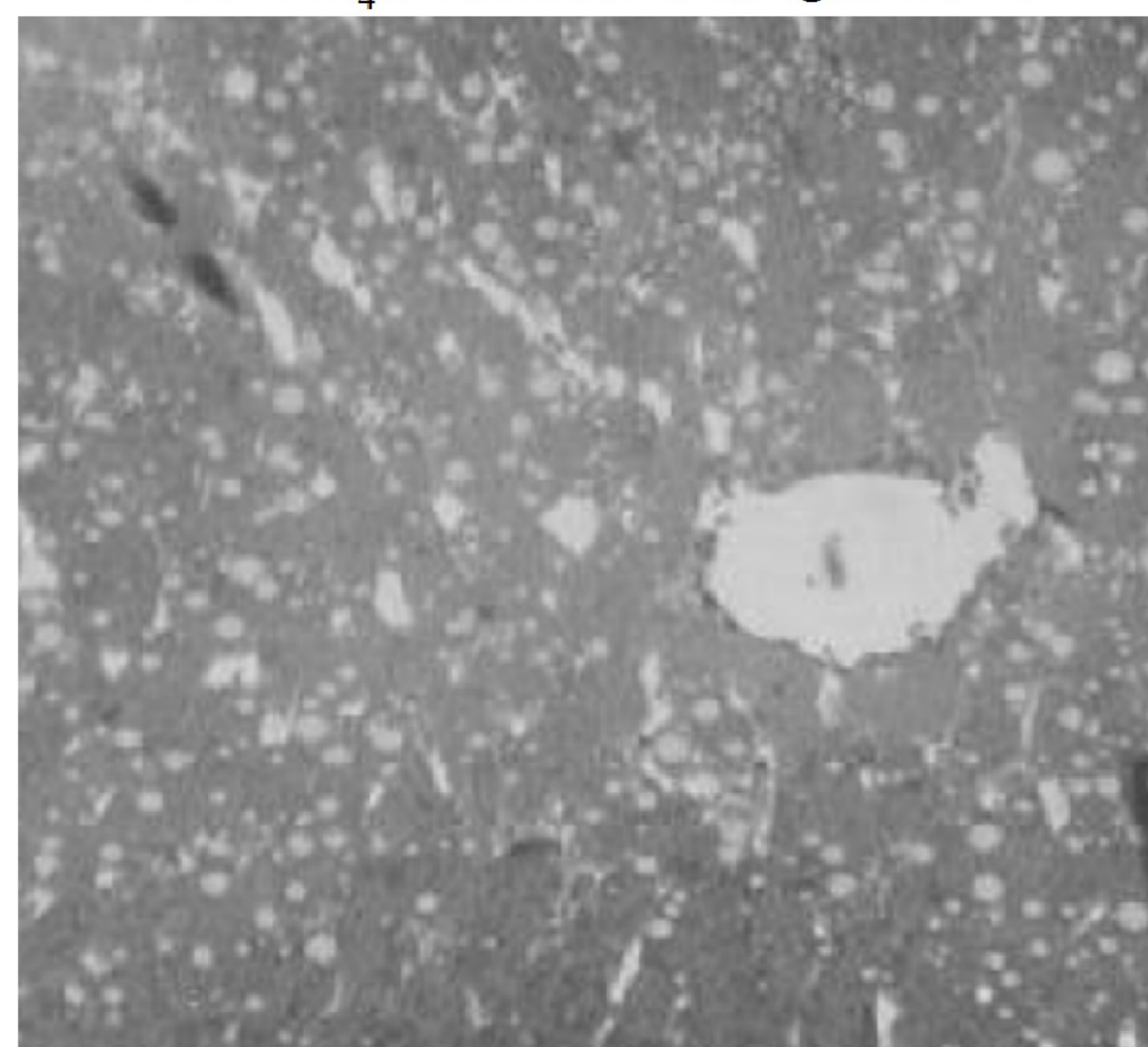


Plate LM 3: Low power light micrograph of rat liver after CCl_4 treatment showing central vein

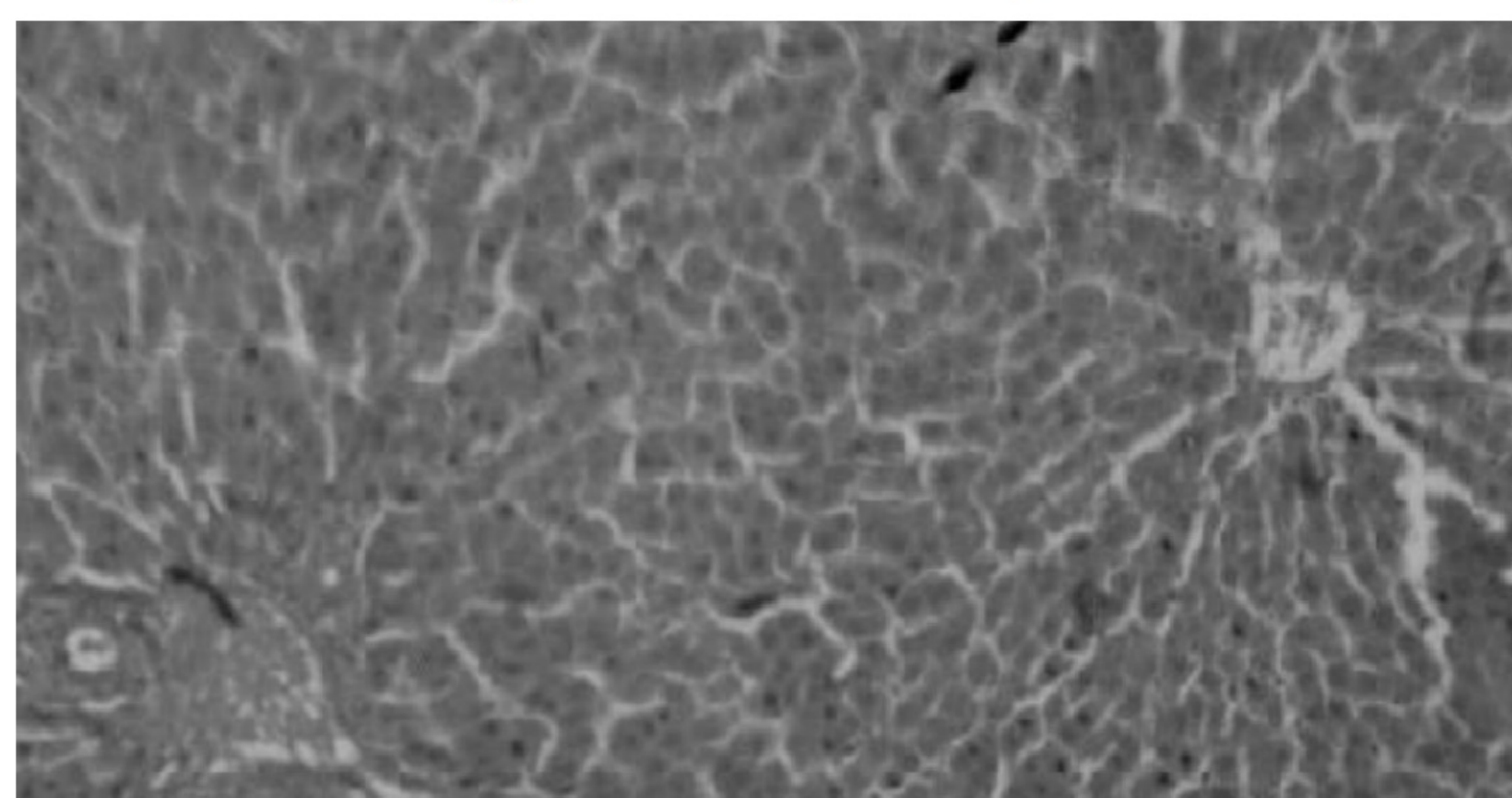


Plate LM 4: Low power light micrograph of rat liver treated with CCl_4 and *Oscimum scantum* plant powder.

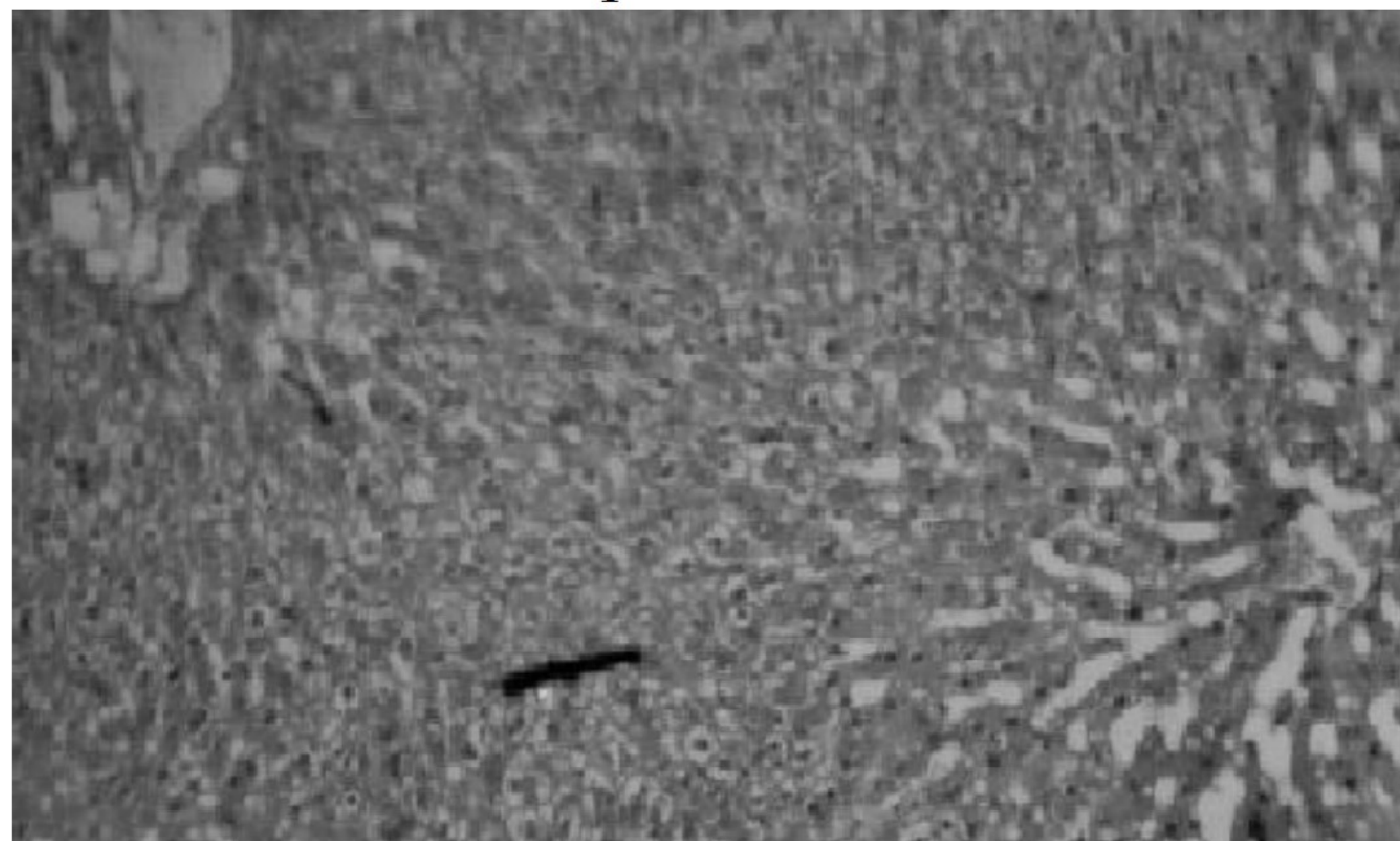
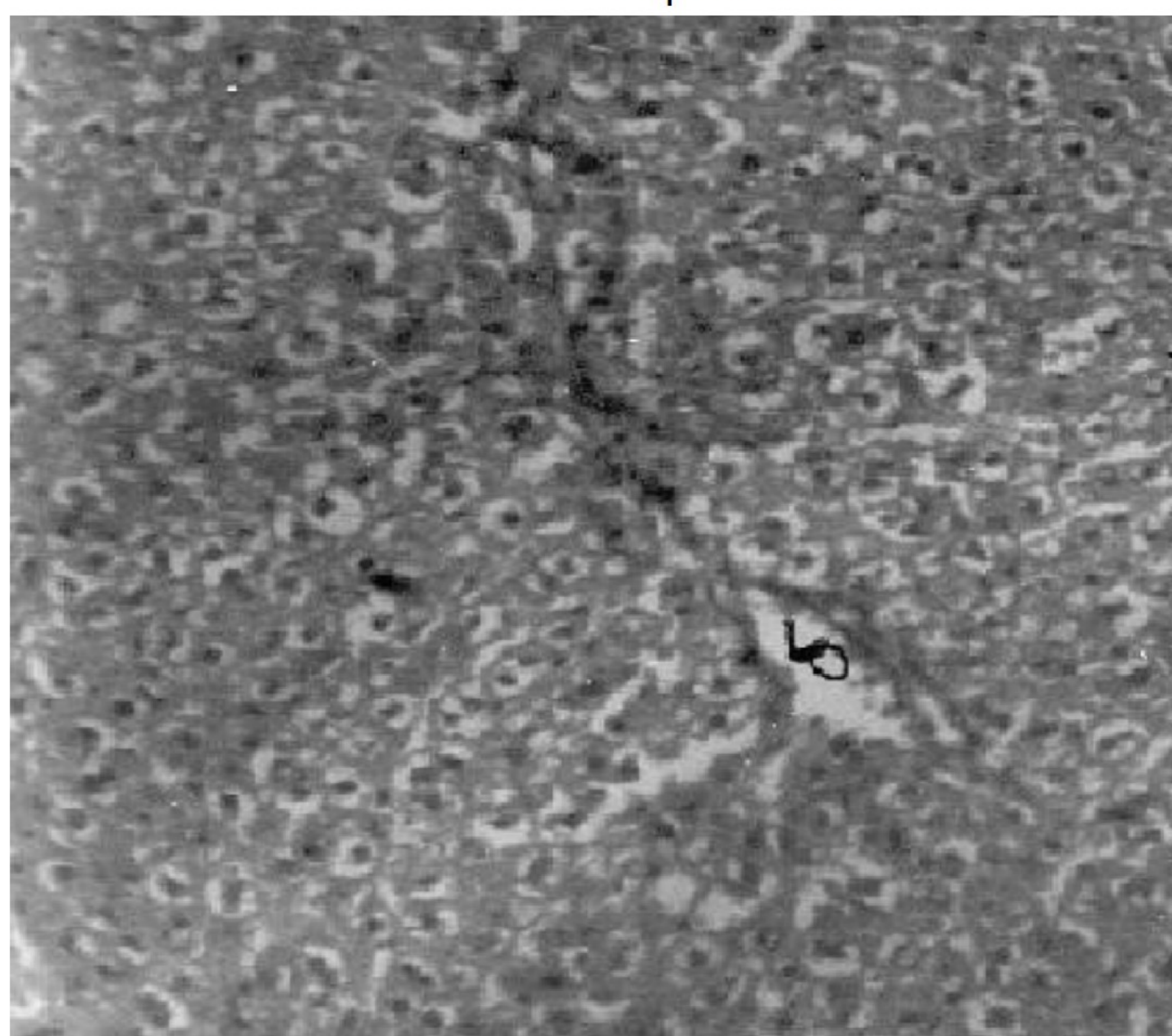


Plate LM 5: Low power light micrograph of rat liver treated with CCl_4 and Silymarin.



4. CONCLUSION

From the results obtained for Group IV animals, it is evident that treatment with the slurry of *Oscimum scantum* brings about significant recovery of the liver. This is supported by the observations in Group III animals where the levels of various liver function parameters have recovered almost to control levels after natural recovery. Cellular recovery after CCl_4 administration takes about 14 days, whereas Group III animals underwent a recovery period of 6 days.

Under light microscope the liver shows distinct centrilobular necrosis after CCl_4 treatment. The hepatocytes in there acinus are vacuolated with distinct dilatation of sinusoids. After *Oscimum scantum*

treatment the CCl_4 damage is reduced, with hepatocytes showing some signs of the recovery. The study thus clearly shows the intrinsic hepatotoxic effect of the plant.

The present investigation therefore adequately proves that *Oscimum scantum* is an effective hepatoprotective agent at the dose (0.50g/Kg) used in the present investigation. The plant slurry impairs normal liver function inducing distinct toxic changes in hepatocytes. This is the dose which show maximum hepatoprotective action against CCl_4 induced liver toxicity. The study reiterates the importance of standardization while formulating herbal based formulation.

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