



COMPARATIVE STUDY ON THE AMELIORATION OF CYPERMETHRIN HEPATIC TOXICITY BY AQUEOUS EXTRACT ON *ANNONA MURICATA* LEAF AND METHOTREXATE IN ALBINO RATS

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ABSTRACT: The present study aimed to examine the comparative study on the amelioration of cypermethrin hepatic toxicity by aqueous extract on *Annona muricata* leaf and methotrexate in albino rats. Wistar strain albino rats of 130-250 grams of body weight (BW) were selected and divided six groups of five rats. First group (Group I) served as normal control. Second group (Group II) given cypermethrin (CYP) (0.05 ml/100g BW/day) for 60 days. Third group (Group III) given aqueous *Annona muricata* leaf extract (AMLE) (0.05 ml/100g BW/day) for 60 days orally. Fourth group (Group IV) was treated with Methotrexate (MTX) (1.0mg/100 gm BW/day) once a week intraperitoneally. Fifth and Sixth group (Group V & Group VI) given cypermethrin (0.05 ml), with supplementations of AMLE (0.05 ml) and methotrexate (1.0 mg/ 100gm BW/week) resp. for 60 days. A significant increase in body weight can be seen in all treatment groups expect MTX treated group, when compared to control, the rate of increase is observed to be almost equal irrespective of treatment on comparison with cypermethrin treated control. On comparison with control a significant decrease in liver weight can be observed in all the treatment groups except MTX supplementation group. But no significant changes can be observed on AMLE supplementation, while MTX supplementation seems to be more beneficial in liver weight. Control group showed normal hepatocytes in trabeculae separated by sinusoids. while no significant pathological changes were observed on AMLE treatment, CYP as well as MTX brought

about an alteration in lobular architecture. Concurrent supplementation was AMLE showed altered lobular architecture with vacuolations and necrosis in hepatocytes, while MTX supplementation showed normal lobular architecture with mild interface hepatitis.

INDEX TERMS - Cypermethrin, *Annona muricata* leaf extract, Methotrexate, Liver Histology and Albino Rats.

I. INTRODUCTION

Cancer, known medically as a malignant neoplasm, is a broad group of varied diseases, all involving cell growth. In cancer, cells divide and grow uncontrollably forming malignant tumours, and invade nearby parts of the body.

The liver is a vital organ and its highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions. Hepatotoxicity implies chemical-driven liver damage. Drug induced liver is a cause of acute and chronic liver disease.

Plants and plant-based medicines are used as the basis of many of the modern pharmaceuticals that we use today in order to treat various ailments. *Annona muricata* is a wide spread small tree, have many biological and pharmacological characters. Likewise anticancer, cytotoxicity, antiparasitic and pesticidal properties are also exhibited.

Cypermethrin an active pesticide belongs to synthetic pyrethroid, it has been used for more than three decades which controls wide variety of pests in agriculture and household applications. In the present study the findings that is to be elicited from this study is the level of production of liver, by the herb *Annona muricata* and comparison with methotrexate (MTX), the anticancer drug, from the destructive role of cypermethrin as a mode of cancer treatment.

II. MATERIALS AND METHODS

2.1 SELECTION OF THE ANIMAL MODEL

Wister strain albino rats weighing about 130-250 grams were selected for the present study. The animals were housed in a well ventilated, temperature and humidity controlled animal house, with a light schedule of fourteen hours and ten hours darkness and were fed with standard diet and drinking water made available at *libitum*.

2.2 PREPARATION OF CYPERMETHRIN (CYP)

Cypermethrin were purchased from the M.Ramasamy Mudaliar and Sons Chemicals Co. CYP was dissolved in corn oil (6 unit cypermethrin and 6 unit groundnut oil) and dosage used is 0.05ml / 100 gm BW once in two days / rat).

2.2.1 PREPARATION OF *ANNONA MURICATA* LEAF EXTRACT (AMLE)

The leaves (*Annona muricata*) were first washed with tap water followed by distilled water and dried under air at room temperature for 24 h. Then these leaves were chopped properly in mechanical blender. The chopped leaves (10 g) were taken in a glass beaker (250 ml) and distilled water (100 ml) was added and mixture was heated to boiling. After 2 h, the heating was stopped and solution colour change was noted from colourless to light brown. The solution was kept to attain room temperature, and then filtered and stored in refrigerator for further use.

2.2.3 EXPERIMENTAL DESIGN

In this present study, healthy male albino rats were divided into 6 groups of 5 animals and received the following regimen of treatments.

GROUP I (C) - Animals received normal saline 1ml/100gm BW/week for 60 days and used as control.

GROUP II (CYP) – Animals were given CYP 0.05ml/100gm BW/week for 60 days orally.

GROUP III (AMLE) – Animals received AMLE 0.05ml/100gm BW/week for 60 days

GROUP IV (MTX) – Animals injected MTX 1.0mg/100gm BW/day once a week intraperitoneally.

GROUP V (CYP+AMLE) – Animals received both cypermethrin (0.05ml/100gm BW/week) along with aqueous *Annona muricata* leaf extract (0.05ml/100gm BW/week) orally for 60 days.

GROUP VI (CYP+MTX) – Animals received both CYP (0.05ml/100gm BW/week) orally along with MTX 1.0mg/100gm BW/day once a week injected intraperitoneally.

All the treatments were given between 9.30 to 10.30 am in the morning. At the end of the treatment protocol, animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in both EDTA coated and uncoated tubes and stored properly. All animals were dissected and their liver was rapidly excised, washed with the saline, blotted with a piece of filter paper and weighed. A bit of tissue from the region of liver were fixed in 10% formalin and used for histological studies.

2.4 STATISTICAL ANALYSIS

Results obtained were tabulated. Statistical analysis was carried out using Dunnetts “t” test. Any significant variation between the control and treated groups were recorded.

III. RESULTS AND DISCUSSION

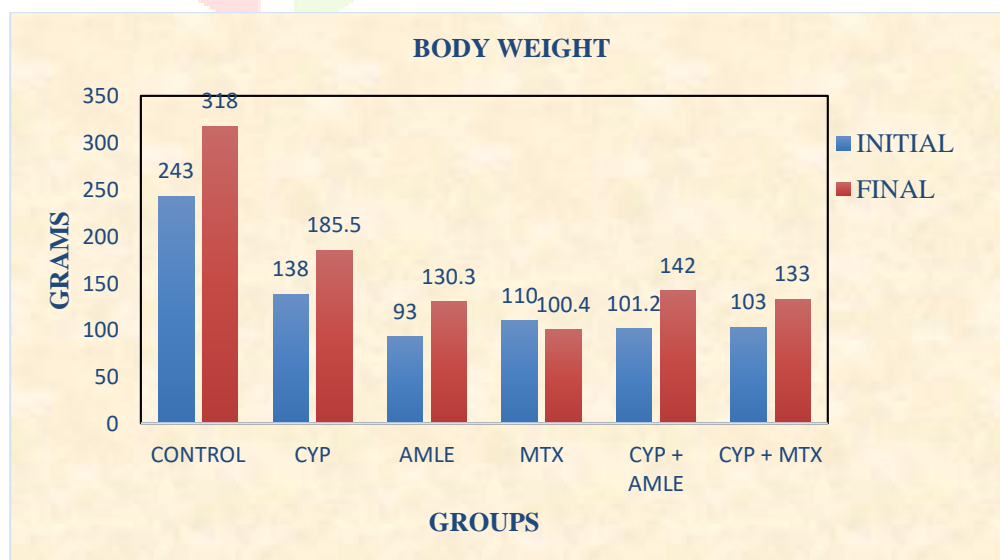
3.1 EFFECT ON BODY WEIGHT (TABLE :1)

Body weight changes serve as a sensitive indicator of general health status animals. The body weight increased and intraperitoneal tissue was significantly reduced by diet containing green tea, caffeine and thiamine. No significant changes on DEN treatment and cinnamon on supplementation in body weight has been reported by Suganya, (2013). Similarly, no significant increase in weight of animals treated with 100mg of AMLE, but significant decrease in percentage body weight on treatment 1000 mg AMLE has been observed by Larbie *et al.*, (2011).

TABLE 1: THE AMELIORATION OF CYPERMETHRIN HEPATIC TOXICITY BY AQUEOUS EXTRACT OF ANNONA MURICATA LEAF AND METHOTREXATE OF BODY WEIGHT IN ALBINO RATS

GROUPS	BODY WEIGHT (Grams)	
	INITIAL WEIGHT	FINAL WEIGHT
CONTROL	243 ± 3.528	318 ± 3.310
CYP	138 ± 1.552*	185.5 ± 0.725*
AMLE	93 ± 1.529*	130.3 ± 1.528*
MTX	110 ± 2.520*	100.4 ± 2.215*
CYP + AMLE	101.2 ± 1.720* ^{ab}	142 ± 2.352* ^{ac}
CYP + MTX	103 ± 2.018* ^{ac}	133 ± 1.327* ^{ac}

Group I- Normal Control, Group II –Cypermethrin, Group III – Leaf Extract of *Annona muricata*, Group IV – Methotrexate, Group V – Cypermethrin+ Leaf Extract of *Annona muricata*, Group VI Cypermethrin+Methotrexate.



Attia *et al.*, (2014) have reported about the significant lowering of body weight on cadmium treatment and reversal of these effects on curcumin supplementation.

In the present study, a significant increase in body weight can be seen in all treatment groups except in methotrexate treated group, when compared to control, the rate of increase is observed to be almost equal irrespective of treatment on comparison with cypermethrin treated control.

3.2 EFFECT ON LIVER WEIGHT (TABLE: 2)

A significant increase in weight of liver on cadmium chloride treatment and subsequent reversal by curcumin supplementation has been reported by Attia *et al.*, (2014). AI- Toae (2014) has also reported about significant increase in liver weight on cadmium treatment and concurrent significant decrease in liver weight on cadmium treatment and subsequent decrease in co-administration with fenugreek seeds, rosemary and cinnamon as well as methotrexate has been reported by Vadivelkumar (2015).

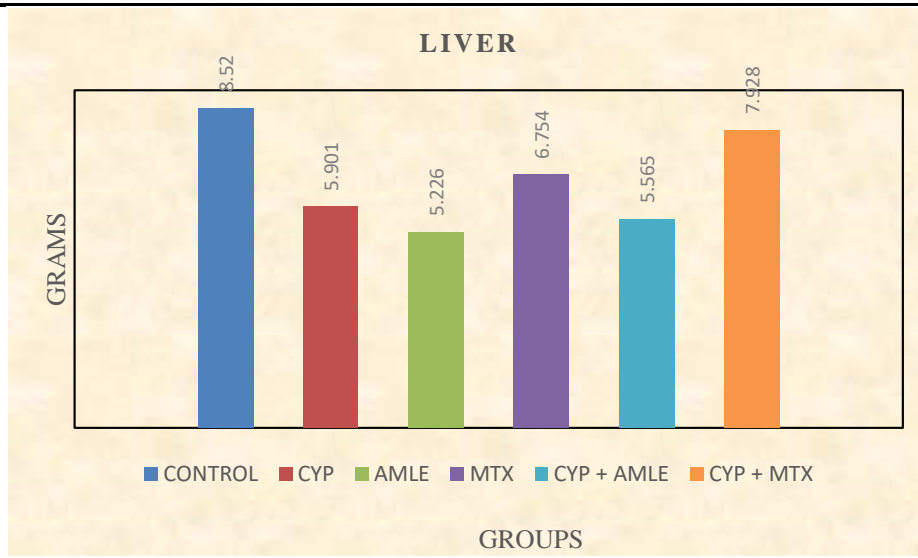
TABLE 2: THE AMELIORATION OF CYPERMETHRIN HEPATIC TOXICITY BY AQUEOUS EXTRACT OF *ANNONA MURICATA* LEAF AND METHOTREXATE OF LIVER WEIGHT IN ALBINO RATS

GROUPS	LIVER WEIGHT (Grams)
CONTROL	8.52 ± 0.123
CYP	5.901 v 0.052 *
AMLE	5.226 ± 0.325*
MTX	6.754 ± 0.194 *
CYP + AMLE	5.656 ± 0.227 * ^a
CYP + MTX	7.928 ± 0.353

Values are expressed as mean ± S.E.M of five rats.

Group I- Normal Control, Group II –Cypermethrin, Group III – Leaf Extract of *Annona muricata*, Group IV – Methotrexate, Group V – Cypermethrin+ Leaf Extract of *Annona muricata*, Group VI Cypermethrin+Methotrexate.

*Significance at 5% level of Group I Vs All groups, ^aSignificance at 5% level of Group II Vs Group III, ^bSignificance at 5% level of Group II Vs Group VI, ^cSignificance 5% level of Group III Vs Group V, ^dSignificance 5% level of Group IV Vs Group VI, ^eSignificance 5% level of Group V Vs Group VI.



But in the present study, a significant decrease in liver weight can be seen in all treatment groups except methotrexate supplementation group, on comparison with control. When compared to cypermethrin control, no significant changes can be observed on *Annona* leaf extract supplementation, while methotrexate supplementation seems to be more beneficial in nature.

3.3 EFFECT ON LIVER HISTOLOGY (FIG: 1)

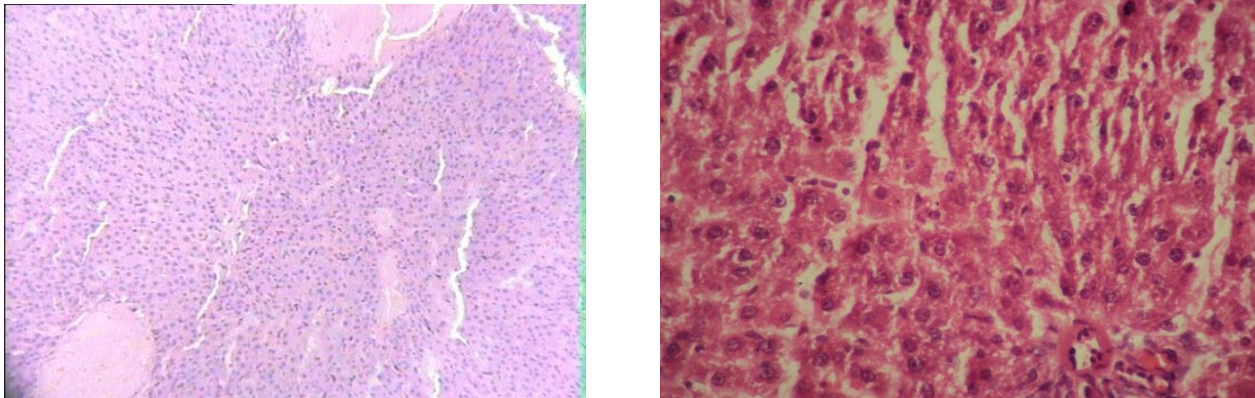
It is known that hepatocytes play a vital role in the proper function of the liver, as they are the main functional cells of the liver. The hepatocytes contain glycogen, and maintain a study level of blood glucose (Junqueira and Carneiro 2003). A compromise in the integrity of the hepatocytes could lead to improper functioning of the liver.

Mahran *et al.*, 2011 have reported the degeneration and increased density of nuclear chromatin with very compact nuclear structure of hepatocytes in cadmium induced rats. Histological changes observed in the photo micrograph of a section of the liver of rats in the hepatotoxic groups showed necrosis and regeneration in the group fed *Gentium africanum* supplemented diet. (Emeka *et al.*, 2010). The aqueous extract of *Psidium guajava* leaves on histopathological study did not show any adverse alteration in the morphological architecture of the liver tissues in both sexes of animal model (Uboh *et al.*, 2010).

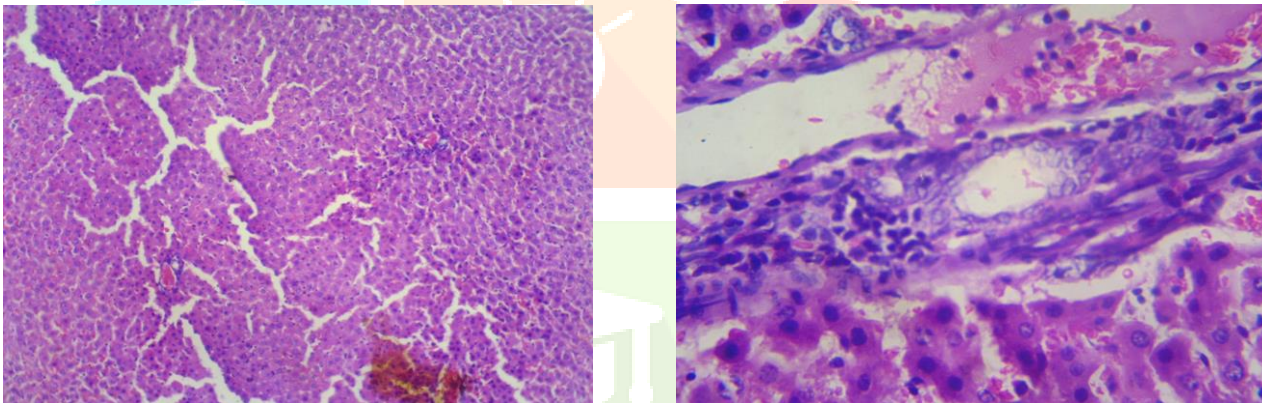
Deranged architecture of hepatocyte with global moderate by hydrophobic degeneration, multiple nucleated cells and deranged sinusoidal arrangement with dilation and vascular congestion have been observed by on AME treatment. (Bitar *et al.*, 2017)

FIG 1: THE AMELIORATION OF CYPERMETHRIN HEPATIC TOXICITY BY AQUEOUS EXTRACT OF *ANNONA MURICATA* LEAF AND METHOTREXATE OF LIVER HISTOLOGY IN ALBINO RATS

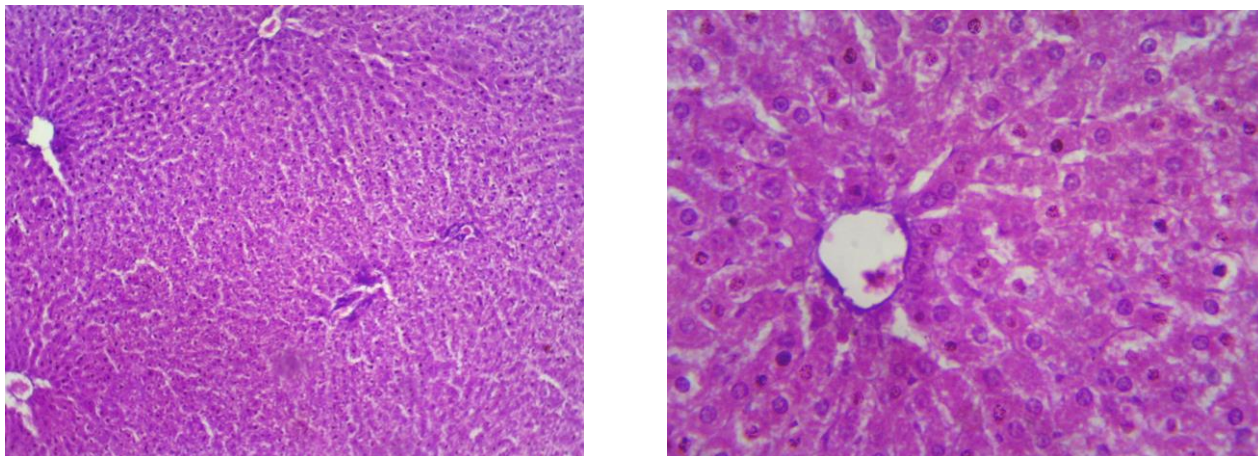
GROUP I – CONTROL LIVER



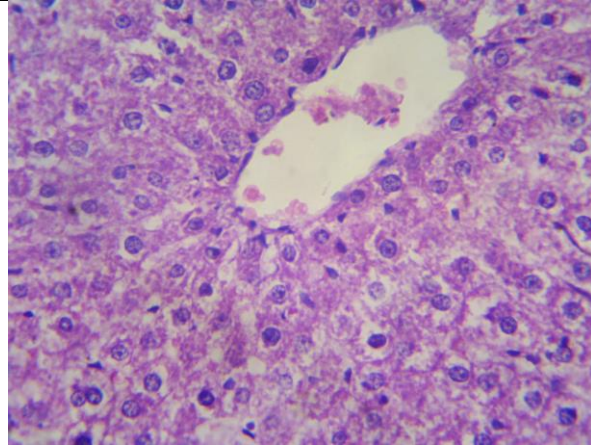
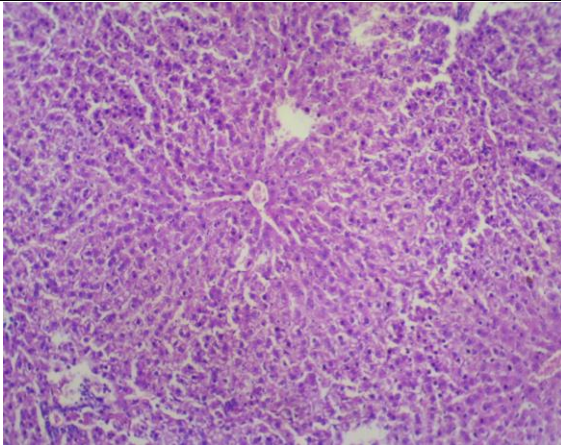
GROUP II – CYPERMETHRIN TREATED LIVER



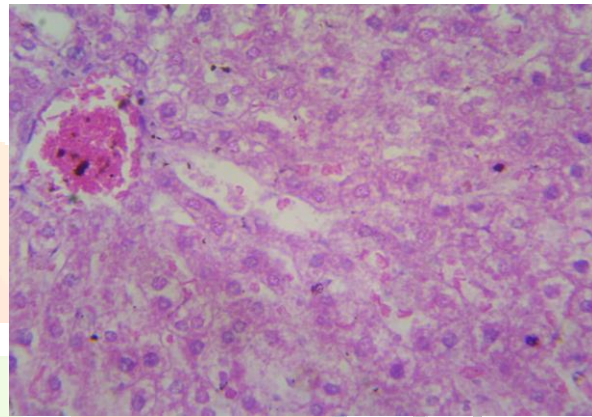
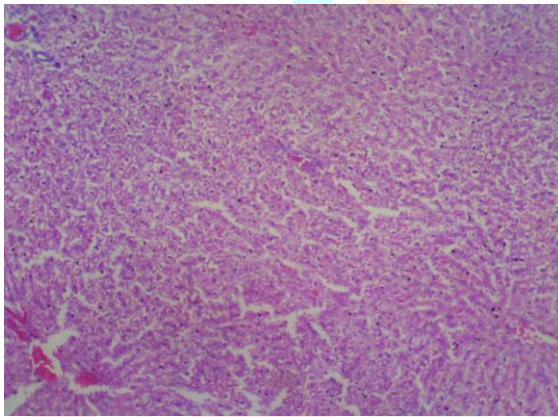
GROUP III – AMLE TREATED LIVER



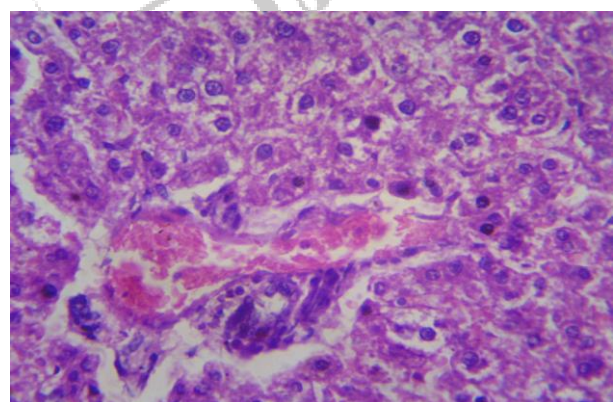
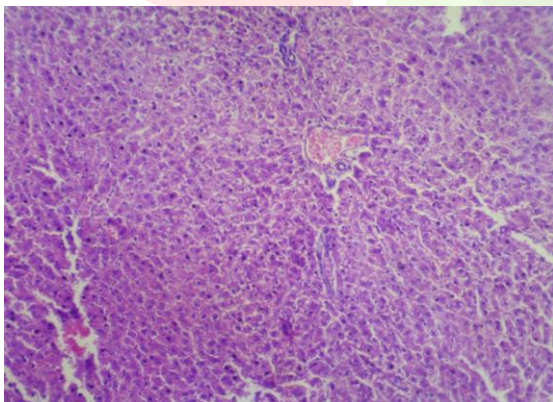
GROUP IV – MTX TREATED LIVER



GROUP V – CYP + AMLE TREATED LIVER



GROUP VI + MTX TREATED LIVER



Section of cypermethrin treated liver showed moderate enlargement of sinusoids, vacuole formation in hepatocytes and central vein congestion. Increase in cypermethrin dose was observed to bring about enlargement of sinusoidal degeneration of hepatic cord, congestion and haemorrhage central vein (Mamun *et al.*, 2014).

In the present study, while no significant pathological changes were observed on AMLE treatment, cypermethrin as well as methotrexate brought about an alteration in lobular architecture. Concurrent supplementation was AMLE showed altered lobular

architecture with vacuolations and necrosis in hepatocytes, while methotrexate supplementation showed normal lobular architecture with mild interface hepatitis.

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