Bioscene

Bioscene
Volume- 22 Number- 03
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Histological Study of the Effect of *Triticum aestivum* on the Liver of Albino Mice *(Mus musculus)* Fed a Fat Rich Diet

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Abstract: Histological changes in liver tissue of albino mice *Mus musculus* was studied using Transmission electron microscopy**.** Liver is the largest gland in the body. The liver Neutralizes and breaks down toxins, drugs, alcohol, and metabolicwastes. It Converts harmful substances (like ammonia, alcohol, and medications) into less toxic compounds for elimination. FRD was prepared by using Edible coconut oil and vanaspati ghee mixture in the ratio of 2:3 respectively and it was administrated at a dose of 10ml/kg body weight with normal chow diet for 30 days. Fresh wheatgrass juice in various dosages was administered. This study used albino laboratory mice (*Mus musculus),* which were 40–50 days old and had an initial weight of 20–40 grams on average. The animals were divided into five groups. After 30 days of the experiment, FRD increases the amount of lipids that accumulate in the hepatocytes' cytoplasm and nucleus. However, following the administration of higher dosages of *T. aestivum*, there are notable alterations, including the elimination of lipid droplets from the nucleus and a decrease in the quantity of lipid droplets from the cytoplasm.

Keywords: Fat Rich Diet (FRD) , *Triticum aestivum*(Wheatgrass) , Liver Tissue, Transmission Electron Microscopy (TEM).

Introduction

Liver is the largest gland in the body. It can be found in the upper parts of the abdomen. The liver is divided into thousands of tiny units called lobules by sheets of connective tissue. A lobule has a central vein in the center with portal triads at the vertices, giving it a roughly hexagonal form. The lobule is the structural unit of the liver (Fawcett *et al.,*2002).Hepatocytes are grouped in hexagon-shaped lobules, also known as classical lobules, which measure roughly 700 μm in diameter and 2 mm in length.Hepatocytes are engaged in the breakdown of xenobiotics, which are proteins that are not native to the body, including poisons and medications. Because they are not hydrophilic, many medications and poisons are not adequately removed from the bloodstream by the kidneys. The liver changes these chemicals into more soluble forms (Nabil, E. M. 2015).

According to research done on mice given a Fat Rich Diet, leads to excessive accumulation of fat in the liver.A Fat Rich diets must include cholesterol as a basic component. Cholesterol is naturally produced by the liver to maintain the body's regular cellular processes. FRD has generally been demonstrated to raise blood cholesterol levels, leading to hypercholesterolemia, a condition that markedly accelerates the development of liver steatosis (Zhang *et al.,* 2021).Hepatic lipid accumulation in hepatocytes, is a significant factor that can cause inflammation, lipid peroxidation, insulin resistance, alterations in energy metabolism, and damage to hepatic cells (Wang *et al.,* 2011).

Triticum aestivum Linn., a common wheat plant in the Poeaceae (Graminae) family, is known as wheatgrass (Suriyavathana *et al.,*2016). According to Rana *et al.,*(2011), wheatgrass is rich in nutrients and low in calories. These nutrients include antioxidants like glutathione, beta-carotene (pro-vitamin A), vitamin C, vitamin E, vitamins K, vitamin B, calcium, iron, magnesium, phytonutrients, and chlorophyll.Wheatgrass contains indole chemicals that raise the activity of the intestinal mucosa and liver's xenobiotic metabolic enzyme, perhaps leading to the deactivation of carcinogens (Bonnesen *et al.,*2001).According to (Devi *et al.*, 2019), wheatgrass has a high quantity of bioflavonoids such apigenin, quercitin, and luteolin, as well as all of the important amino acids, primarily alanine, aspartic acid, glutamic acid, arginine, and serine, which help the body produce enough protein. It is capable of ferric reduction and superoxide scavenging. Superoxide dismutase (SOD) helps to digest the toxin by reducing the effects of radiation. (Bar-Sela *et al.,*2007; Cao *et al.,*1996).

Materials and methods

Plant materials

In this experiment, wheatgrass *(Triticum aestivum*) was cultivated in the zoology garden of the P.G. department. The grass was trimmed ½ inch above the soil's surface after it reached a height of roughly 6 inches. A grinder was used to crush twenty grams of freshly picked grass with 10 ml of sterile water. The juice was then extracted using four layers of damp muslin cloth. The filter was made to a final volume of 20 ml using sterile water, and it was administered as grass juice every day. Before administration, a fresh extract was made.

Method of Preparation of Fat Rich Diet

According to the procedure of (Shyamala *et al.,*2003), edible coconut oil and Vanaspati ghee were purchased from the market and combined in a 2:3 v/v ratio. For 30 days, it was administered at a dose of 10 ml per kg of B.W along with a regular chow diet.

Experimental Design

Albino laboratory mice (*Mus musculus*),40-50 days old, average initial weights 20-40gm, was be used in this study. These animals was kept in Polypropylene

cages under standardized conditions of temperature and light in animal house of University Department Of Zoology, T.M.B.U. The animals were divided into 5groups . Doses were given for 10 days. All the animals were taken care of under ethical consideration and the experimental protocol.

Each group received treatment as follows:

Group A : Control Group

Group B: Fat Rich Diet (10ml/kg B.W/day)

Group C: FRD((10ml/kg B.W/day) along with *T. aestivum* (10ml/kg B.W/day)

Group D: FRD((10ml/kg B.W/day) along with *T. aestivum* (20ml/kg B.W/day)

Group E: FRD((10ml/kg B.W/day) along with *T. aestivum* (50ml/kg B.W/day)

Transmission Electron Microscopy

To ensure correct fixation, dissect the organs or cut liver tissue samples into 2 x 2 mm pieces . Samples are fixed for four to six hours at 40 degrees Celsius in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. Keep the fixative outside of the refrigerator until it reaches 20 degrees Celsius before fixation. Samples are first fixed for 15 to 20 minutes in this non-cold fixative, and then they are refrigerated for 4 to 6 hours. Every hour of fixation, agitate the sample vials with the fixative for two to three minutes.Wash in buffer for 3 times, each for one hour duration at 40C.

Results

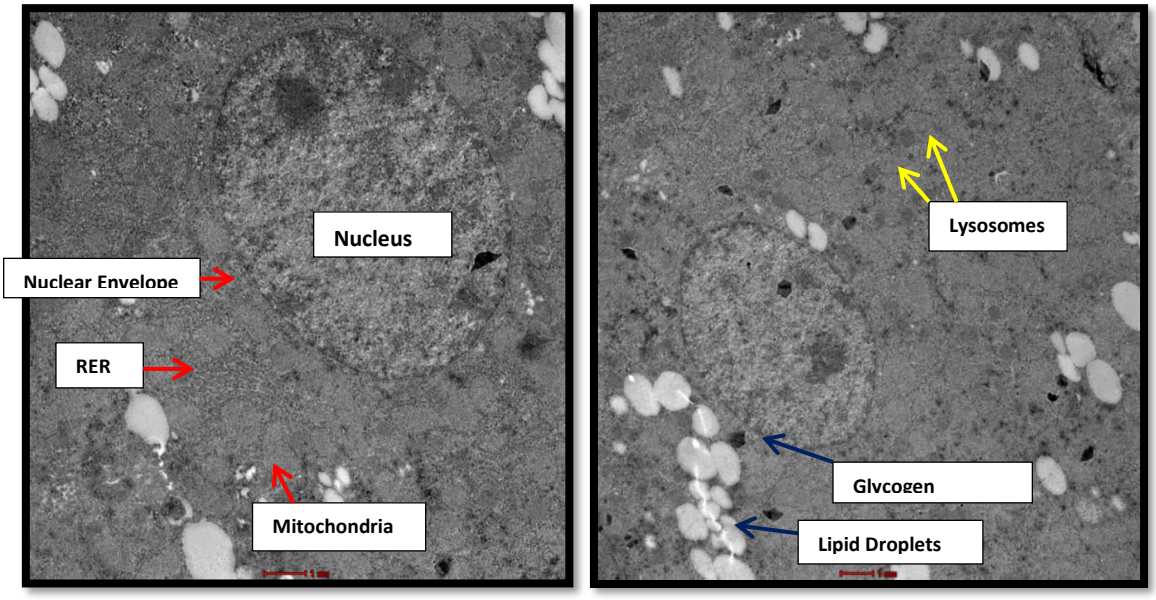

Fig1 :-TEM of Liver Tissue of albino mice of Control Group A

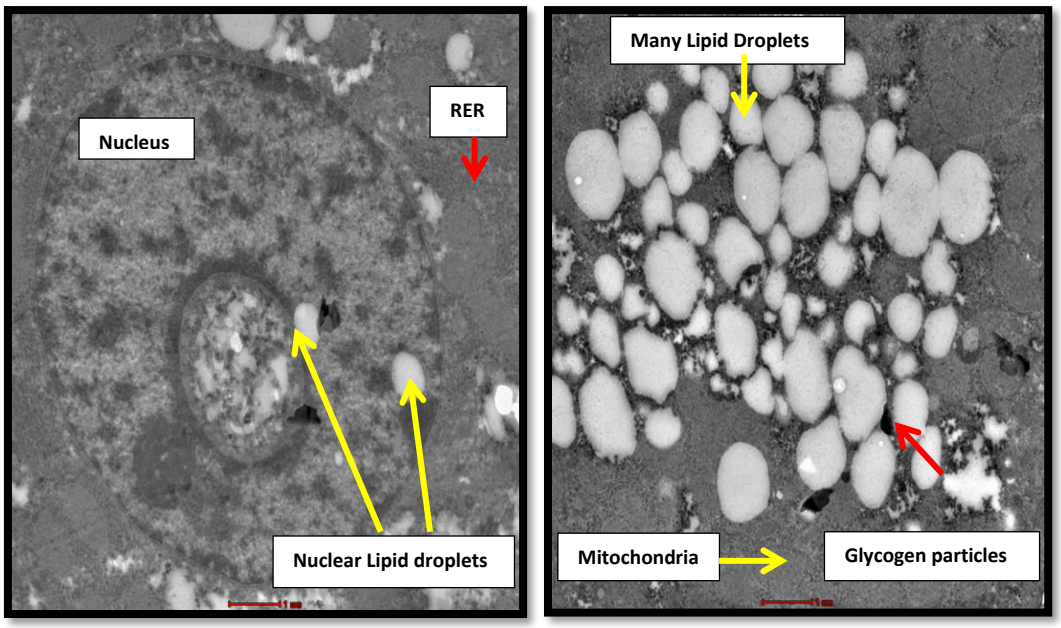

Fig2 :-TEM of Liver Tissue of albino mice ofGroup B Treated with FRD.

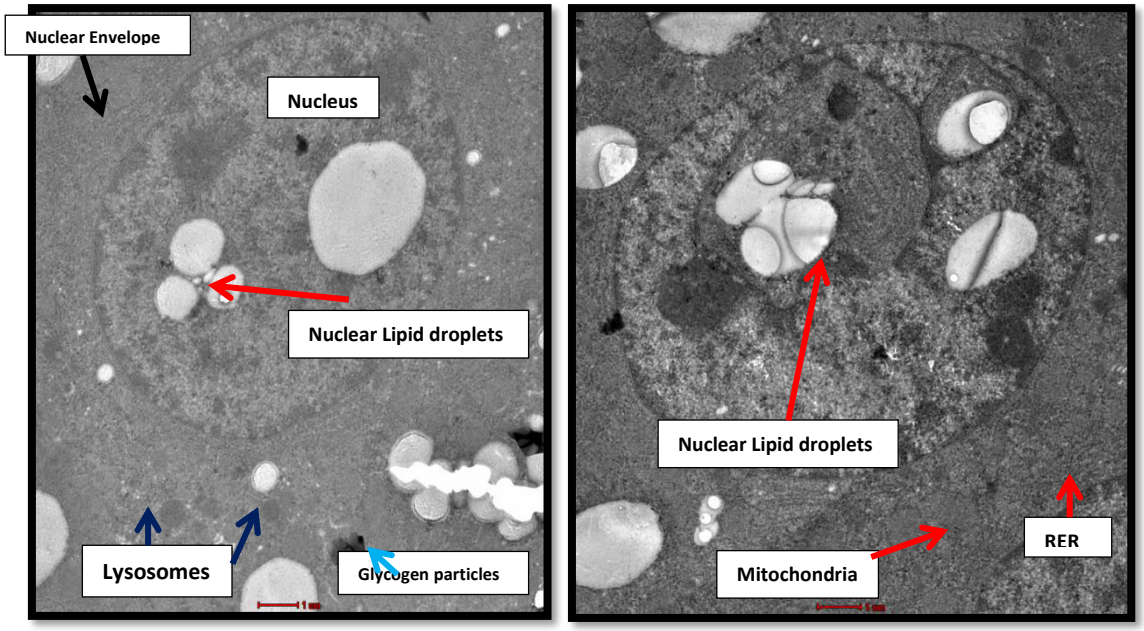

Fig3 :-TEM of Liver Tissue of albino mice ofGroup C Treated with FRD along with *Triticum aestivum* (10ml/kg B.W./day).

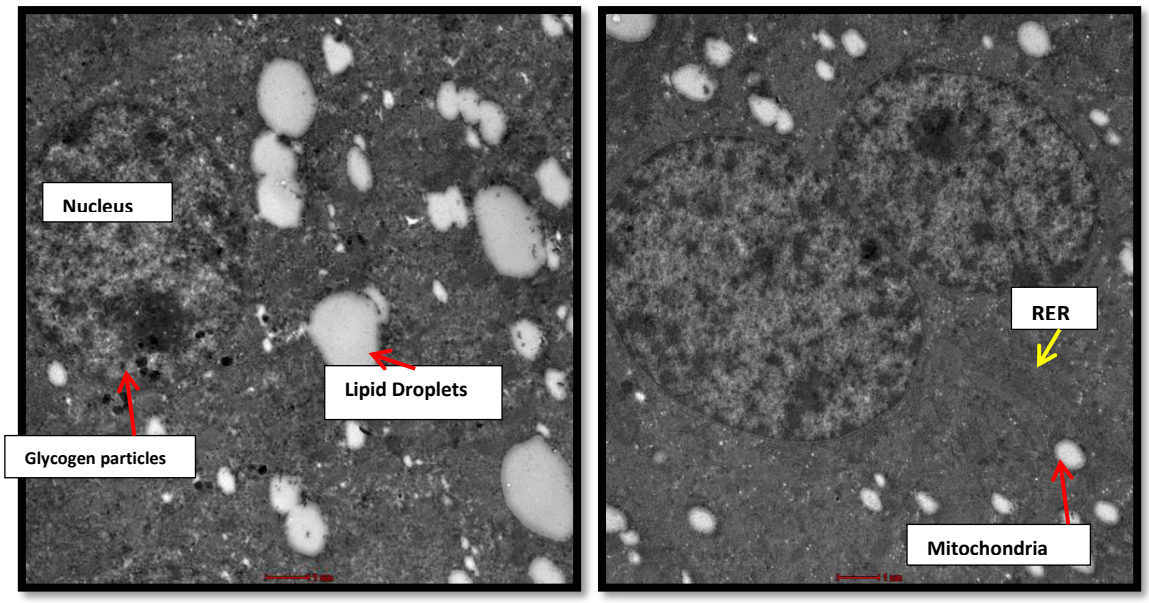

Fig 4:- TEM of Liver Tissue of albino mice of Group D Treated with FRD along with *Triticum aestivum* (20ml/kg B.W./day).

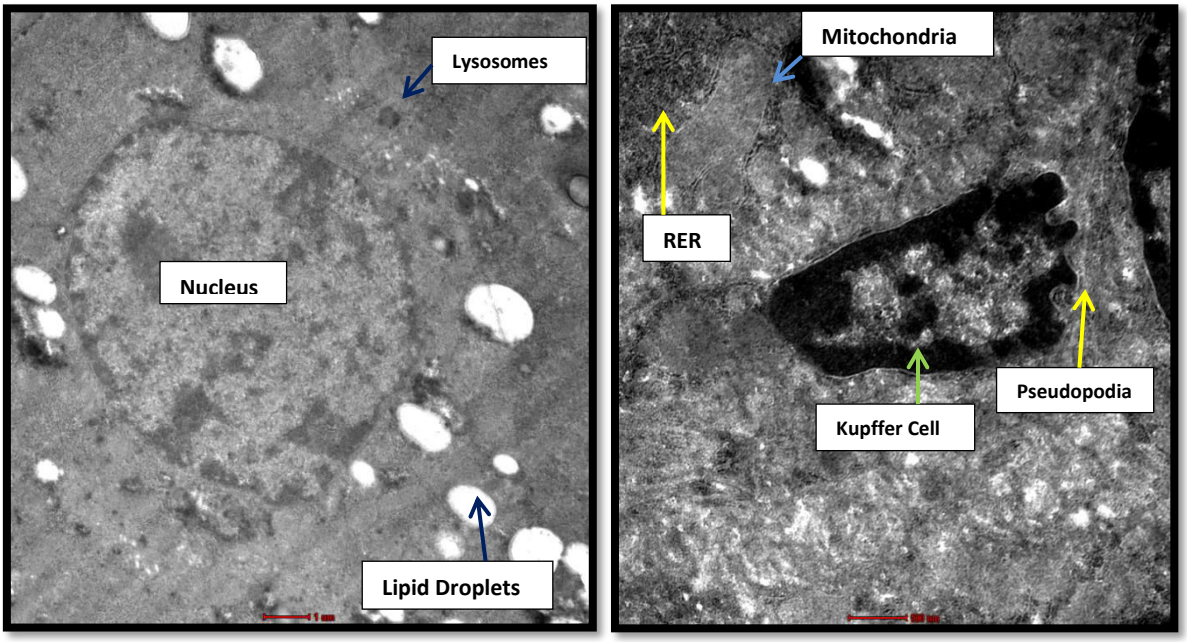

Fig 5:- TEM of Liver Tissue of albino mice of Group E Treated with FRD along with *Triticum aestivum* (50ml/kg B.W./day)

Discussion

Histologically study of TEM of the control group of liver cell hepatocyte was composedof round nucleus, lysosomes, canaliculi glycogen particles, lipid droplets andmany kupffer cells. Cytoplasm has abundant RER , mitochondria.

Albino mice treated with only Fat Rich diet in group B shows the abundant lipiddeposits within cytoplasm of the hepatocytes and also nuclear lipid dropletspresent. Hepatocyte contains round nucleus, mitochondria , glycogen particles,RER and kupffer cell.

In Group C, hepatocytes has nuclear lipid droplets, cytoplasmic lipid droplets,round nucleus, mitochondria , glycogen particles, RER, lysosomes and kupffercell.

In group D and E hepatocytes do not have nuclear lipid droplets , low amount ofcytoplasmic lipid droplets, round nucleus, lysosomes , kupffer cell , abundantmitochondria and RER , glycogen particles, RER.

Fat and cholesterol absorbed from the diet were brought to and accumulated inthe liver which produces fatty acids and cholesterol from acetyl-CoA comefrom glucose. During starvation, extra fatty acids may be transformed in theliver into ketone bodies, which provide energy to the brain and muscles(Chiang, J. 2014). The liver uses the absorbed and adipose tissue-derived freefatty acids (FFAs) for energy production, membrane synthesis, or triglyceride(TG) storage (Bazinet *et al.,*2014).Within the hepatocytes, FFA attaches itself to fatty acid binding protein(FABP)-1, which transports FFAs from the cytoplasm to various cell compartments for either metabolism or gene expression regulation byinteracting with transcription factors like peroxisome proliferator-activatedreceptor (PPAR)α, thereby shielding the liver from lipotoxicity (Chen *et al.,*2006)

(Shi *et al.,*2012) came to the conclusion that "serum FABP1 correlated withobesity, insulin resistance, hypertriglyceridemia, and low HDL cholesterol"after conducting their study on Chinese individuals. These results suggested thatFABP1 plays a critical function in both hepatic cell protection and lipidmetabolism in the liver.It has been revealed that adipocytes-FABP (A FABP), which influences insulin sensitivity and the levels of non-esterified fatty acids in the blood, also affects lipid metabolism in the liver. In comparison to obese control mice, it was reported that "mice lacking A-FABP in adipocytes showed reduced lipolysis and were more insulin sensitive." (Uysal *et al.,*2000)

In the present histologically study of liver I was found that Albino mice treated with Fat Rich diet shows the abundant lipid deposits within cytoplasm of the hepatocytes and also nuclear lipid droplets present.

(Ha *et al.,*2023) analysis liver histology and revealed that High Fat Diet (HFD) caused normal steatosis, as evidenced by many lipid droplets and ballooning degeneration. HFD led to an increase in adipose tissue mass and dyslipidemia. Adipocyte hypertrophy brought on by elevated serum TG, TC, and free fatty acid levels is primarily responsible for this. A study conducted on mice given HFD showed that hepatocyte-specific CD36 disruption enhanced insulin sensitivity and fatty liver states (Wilson *et al.,*2016). CD36, a free fatty acid (FFA) transporter gene, was upregulated by ~29fold in the HFD-fed group (Koonen *et al.,*2007).

(Wang *et al.,*2008) reported that the rat feed with High Fat Diet causes increased levels of caspase-3 and apoptotic hepatocytes which are linked to elevated oxidative stress of hepatic cells.According to the study of (Gadallah *et al.,*2023) When a high-fat diet was consumed by the mother, the liver cells of the mother rats and their progeny underwent significant biochemical, histological, and ultrastructural alterations.

The present study also revealed that in group D and E albino mice treated with FRD along with higher dose of *T. aestivum* shows hepatocytes do not have nuclear lipid droplets , low amount of cytoplasmic lipid droplets.

Numerous enzymes in wheatgrass juice help the body rid itself of toxins and pollutants, and amino acids are a vital part of this natural cleanser, which helps the liver be detoxified, remove harmful heavy metals from the bloodstream, remove waste from the body, and slow down the aging process (Sareen *et al.,*2014). Choline inhibits the accumulation of fat. In the same way, magnesium aids in the extension of excess fat. Potassium stimulates and energizes, while magnesium sulfate extracts pus from an illness. Indole is another flavonoid found in wheatgrass that aids in the liver's development of enzymes and the deactivation of carcinogens (Wigmore, A., 1985).

It has been observed that the bioactive plant compounds triterpenoids and flavonoids can alter lipid levels (Koshy*et al.,*2001 and Zhang *et al.,*2008). According to (Tebib *et al.,*1994), tannins are said to increase the activity of the endothelium-bound lipoprotein lipase activity, which hydrolyzes triglycerides.

According to several research, when wheatgrass extract was added to a rat model, the level of liver enzymes restored to nearly normal. Wheatgrass contains chemicals called chlorophyll and choline, which are known to be the primary nutrients responsible for liver protection. Vitamins like potassium and magnesium found in wheatgrass keep the liver healthy and stop fat from accumulating there (Mishra *et al.,*2025).

Choline acts as detoxifying agent and helps removal of esters of cholesterolsand sterols. Choline also has been showed lipotropic action and promotes transportation of fatty acid in plasma and removes lipid from liver, thereby preventing accumulation of fat in the liver (Qamar *et al.,*2018). Wheatgrass's chlorophyll is said to be a natural bodily cleanser due to its ability to help the body eliminate toxins and other harmful compounds, as well as to help remove drugs and other potentially harmful foreign elements(de Vogel *et al.,*2005).

Conclusion

After 30 days of experiment FRD causes increases the accumulation of lipids in cytoplasm as well as in nucleus of the hepatocytes. But after Higher doses of *T. aestivum* administration shows significant changes and disappearance of the lipid droplets from nucleus and also reduction of lipid droplets amount from cytoplasm.

Acknowledgement

We are grateful to the university Department of Zoology, T.M.B.U , Bhagalpur, Bihar and The Officer-In-Charge Sophisticated Analytical Instrument Facility Electron Microscopy Facility, Department of Anatomy Basement Lab (-1 floor), Convergence Block AIIMS, New Delhi provided all of the lab equipment required

to finish this experiment. We also thankful to Gaurav Kumar and Divyanshu Kumar Research Scholar for their support during the experiment.

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