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IMPACT OF CAFFEINE ON THE HISTOLOGY OF LIVER AND BLOOD MICROFLORA OF ALBINO RATS.

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Abstract

Caffeine is one of the most widely consumed as well as pharmacologically active ingredient in the world. Traditionally use of caffeine has increased substantially as many caffeinated drinks are being consumed. The main effects of caffeine are anxiety, fast heartbeat, restlessness, insomonia and dehydration. The present study analysed the histology of liver and effect on blood microflora of albino rats. The liver plays an essential role in metabolism and produces digestive enzymes stores bile produce hormones and remove toxins. Three doses were tested to observe histological changes in microflora of blood of animals, they were 3 mg/5ml distilled water; 6 mg/5 ml distilled water and 9 mg/5ml and extreme dose of 125 mg/5 ml distilled water. Untreated rats were kept as control. There were three replicates of each treatment. In animals treated with 3 mg/5 ml of distilled water showed rounded hepatocytes with ill-defined cell walls. Transverse section of liver treated with 6 mg/5 ml of distilled water showed rounded occlusion of central vein with deformed RBC's and leucocytes, most macrophages had empty spaces at the site of central vein, while in the acute dose of caffeine thrombosis of blood vessels, aggregation of platelets, bound together by erythrocytes and fibrin. The leucocytes were visible in large blood vessel. Nuclei of hepatocytes disappeared while in the untreated albino rats the liver tissue was normal. While 9 mg/ 5 ml distilled water showed thrombosis, aggregation of platelets bound together by fibrin, while acute dose revealed degeneration of hepatic cells. Endothelial cells were abnormally enlarged and necrosis.A regular blood culture was done to detect any becteremia. Blood cultures of treated and untreated animals were tested. Blood of treated and untreated albino rats did not reveal the presence of bacteria i.e. no bacteremia.

Keywords: Caffeine, Albinorats, Liver, Blood Culture, Histology.

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INTRODUCTION

Caffeine is a pure alkoid trimethylxanthine is present in tea, coffee and popular energy drinks (Barone and Roberts, 1984). It stimulates the activity of brain and nervous system. Generally people use it for the mental alertness, atheletic performance, for headache, memory loss and obesity.

Caffeine is unsafe when taken in high dose for a long period of time and may cause restlessness, increased heart rate, nervousness, chest pain, anxiety, specially people suffering from hypertension, (Pohanka and Dubes, 2013)

Liver plays an important role in the elimination process of body wastes in all living organisms, therefore any damage to liver results in accumulation of body toxins and metabolic dysfunction (Baravalia *et al.,* 2011, Rodak *et al.,* 2021). Caffeine consumption first step is biotransportation is inverted by hepatic microsomal enzymes and selective catalysis by cytochrome P450PA in liver, (IARC, 1991). In some studies it has been suggested that free radicals were produced by caffeine which may result in increase of lipid preoxidation by increasing oxidative pressure (Vistisen *et al.,* 1992; Dianzani *et al.,* 1991).

Guth *et al.,* (2022) reported that caffein increases the function of urea cycle in liver Augmenting ammonia degradation antagonizing adenosine receptor it also has hepatoprotective change forthcoming formation of Fibrosis.

Munawar *et al.,* (2016) found that caffeinated drinks influenced morphology and histology of liver of albino rats.

Soffritti *et al.,* (2002); Clauson *et al.,* (2008) reported that prolonged use of caffeine resulted in malignant pancreatic carcinoma and mammary tumor, besides jaundice, hepatitis and hypertension. The present study analysed the effect of different doses of caffeine on the histology of liver and influence on blood microflora of albino rats.

MATERIAL AND METHODS

The albino rats (male) were obtained from the University of Karachi's animal house, housed in cages of plastic. The room were well ventilated and the rats received regular human care according to the protocol allowed by the Zoology Department committee. The weight of albino rats was 185-200 gms, they were daily supplied with pelleted rat chow and distilled water. Lahore pharma provided caffeine citrate. The rats groups were given 3 mg, 6 mg, 9 mg and 125 mg/ 5 ml distilled water. Different doses to the groups were given via oral route through a sterile gastric feeding tube that it would not harm the trachea. The control animals simply received distilled water. Once animals started dying fresh liver sections were obtained from the rats and paraffin sections 8 µm.were prepared for histological study. In rats alive incision was made in the abdomen and incised upto neck in the dead animals and selected for histological procedure. Haemotoxylin and eosin were used for staining and finally photography was done using an automatic photographic camera mounted on a microscope (Nickon optical).

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In order to check any bacteria in blood regular blood cultures was conducted the microbiological detection was done according to Wistreich and Lechtman, (1988). The cultures used for blood samples were nutrient agar (E20 laboratories ltd) and blood agar (Oxoid). The agar plates were incubated for 24 hours at room temperature range (38-41°C) in Laboratory of Microbiology department, University of Karachi.

RESULTS

The albino rats treated with various doses of caffeine showed that higher dose severe damage to the liver was caused unlike the healthy liver which had clear morphology (Fig. 1).

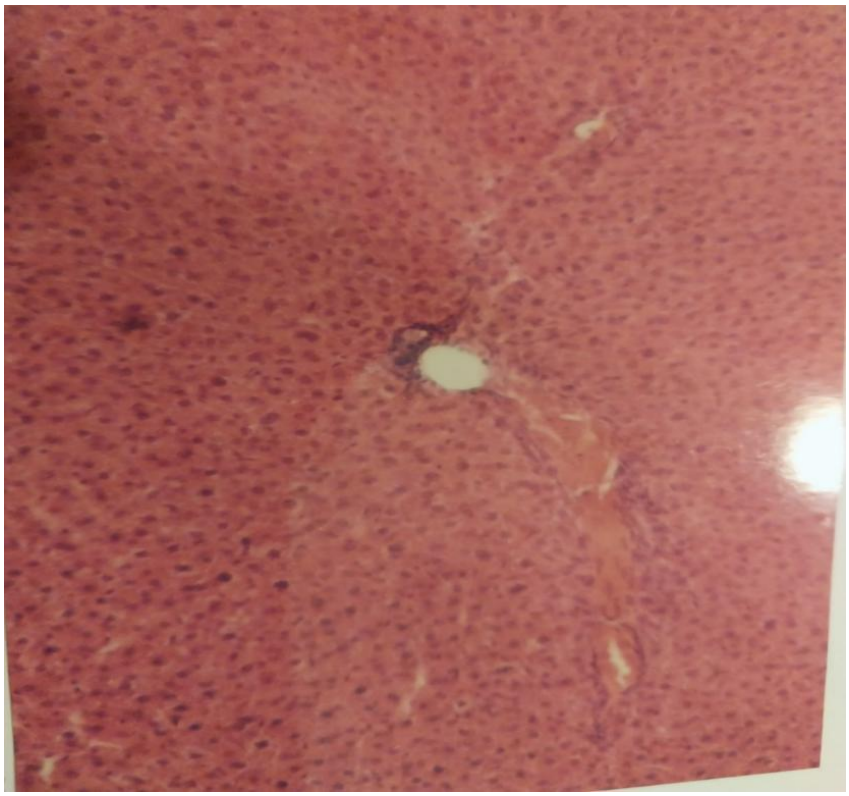

Figure 1: Transverse Section of the Normal Histology of Liver Tissue of Albino Rat (x50) (H2E stain)

At 3 mg/100 ml distilled water dose liver showed slight changes but still the architectural condition were identifiable (Fig. 2) except heptocyte walls were ill defined but at higher dose 6 mg/5 ml sinusoids occulsion of central veins with deformed red blood cells and leucocytes, mostly microphage, leaving an empty space at the side of central vein (Fig. 3). The group treated with 9 mg/100 ml of caffeine showed thrombosis of blood vessels, aggregation of platelets, bound together by fibrin and erythrocytes. The leucocytes could be observed in the large blood vessels (Fig. 4), while at higher magnification enlargement of the endothelial cells was very prominent.

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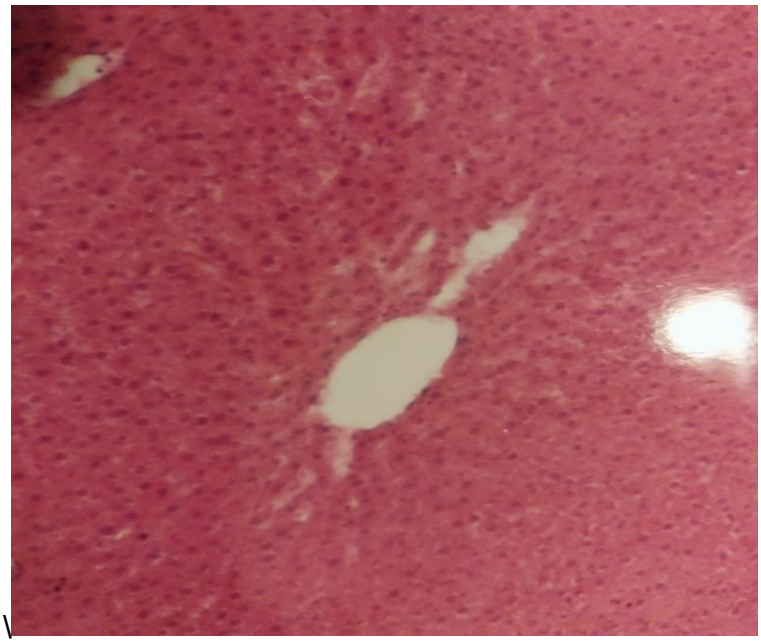

Figure 2: Enlarged Transverse Section of the Liver Tissue of Orally Treated Albino Rat with 3mg/5ml of Caffeine. Hepatocyte Walls are ill defined (X200) (H2E Stain)

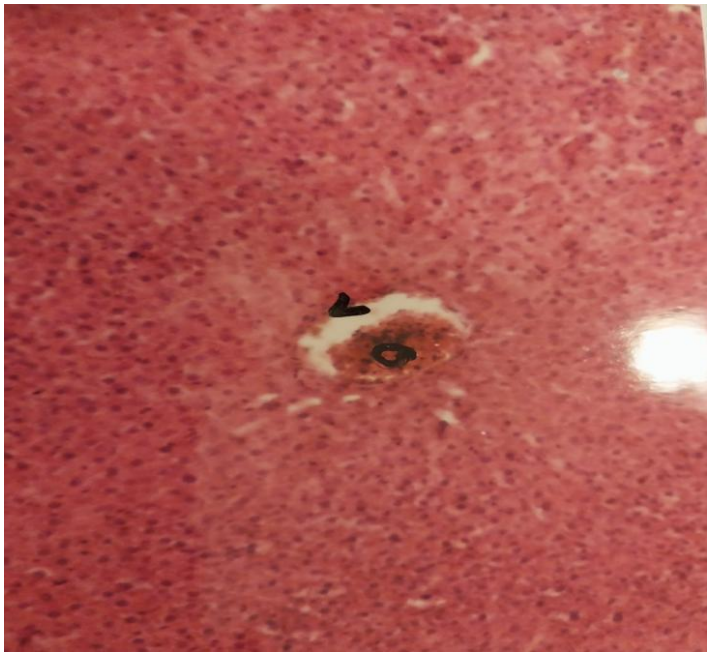

Figure 3: Transverse section of the liver tissue of orally treated albino rat with 6mg/5ml of caffeine showing occlusion (O) of central vein (V) with deformed red blood cells and leucocytes, mostly microphage, leaving a small empty space at the site of the central vein. (x50) (H2E Stain)

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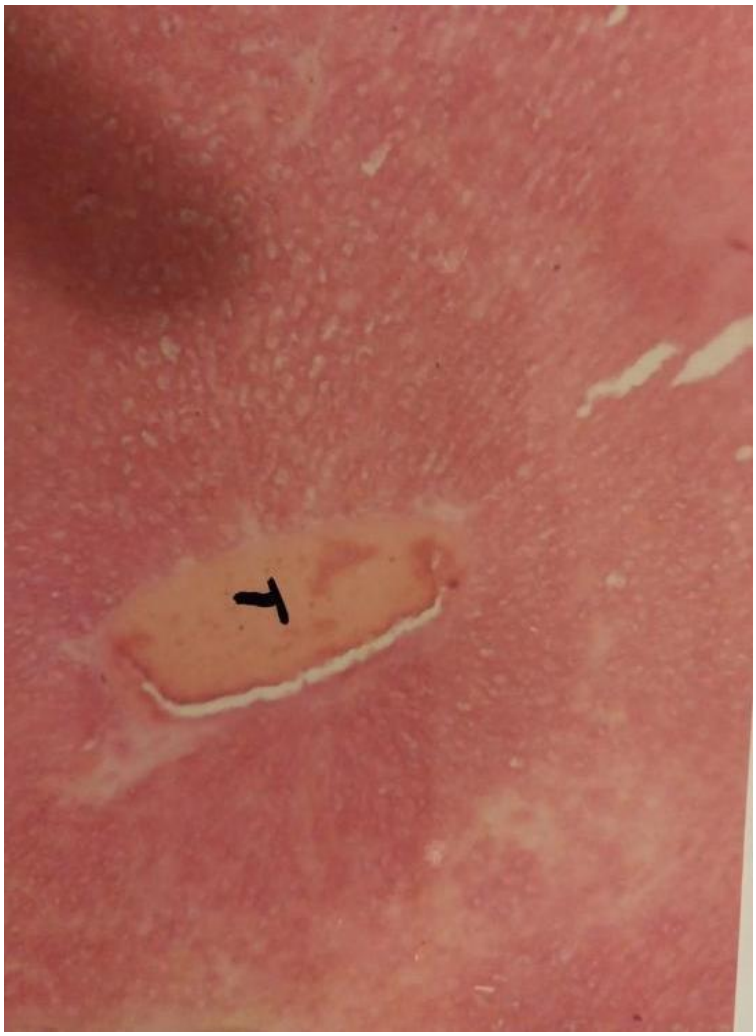

Figure 4: Transverse section of the Liver Tissue of albino rat with 9mg/5ml of caffeine showing thrombosis (T) of blood vessels, aggregation of platelets, bound together by fibrin and erythrocytes. Leucocytes are visible in large blood vessels (x50) (H2E stain)

Nuclei of hepatocytes cell had disappeared. Slugging in the central vein had almost closed the lumen except a narrow passage (Fig. 5).

The group treated with acute dose 125 mg/100 ml of caffeine showed necrosis and degeneration of endothelial blood vessels.

The degeneration of hepatic cells was cloudy at the same time endothelial cells of sinusoids are abnormally enlarged and the hepatocytes looks like a mass of homogenous material with traces of nuclei but without any cellular demarcation (Fig. 6).

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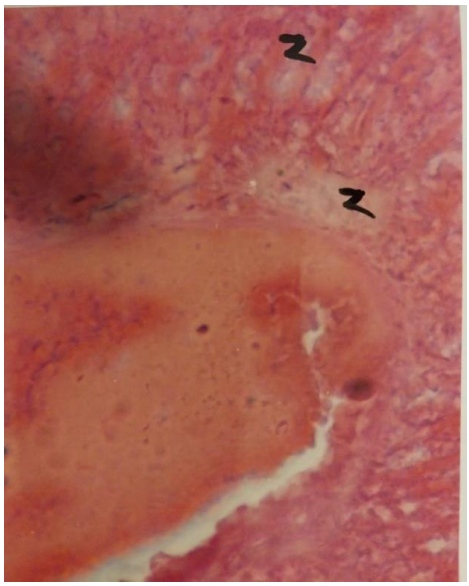

Figure 5: Enlarged transverse section of the liver tissue of orally treated albino rat with 9mg/5ml of caffeine showing enlargement of endothelial cells. Nuclei (N) of hepatocytes cells have disappeared. Slugging in the central vein is prominent almost closing the lumen except a narrow passage on one side (X200) (H2E stain)

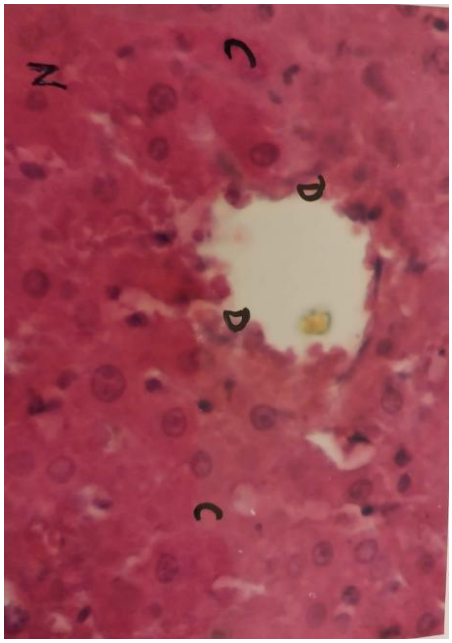

Figure 6: Liver Tissue of albino rat with 125 mg/5ml caffeine necrosis (N), degeneration (D) of endothelial blood vessels. Cloudy (C) degeneration of hepatic cells are prominent. Endothelial cells of sinusoid are abnormally enlarged and the hepatocytes look like a mass of homogenoeous material with traces of nuclei but without cellular demarcation (x50) (H2E stain)

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The microbiological analysis of blood test for control and treated animals revealed no bacteria growth i,e no bacteremia was detected and blood sample of treated animals was similar to control.

Table 1: Microbiological Findings of Blood Samples of Rats Treated with different Doses of Caffeine

Media	Animal groups				Organisms detected
	3mg	6mg	9mg	125 mg/5 ml distilled water	
NA	-	-	-	-	Nil
BA	-	-	-	-	Nil

NA: Nutrient Agar BA: Blood Agar

There were three replicates of each observation.

DISCUSSION

Caffeine, a purine alkaloids (1, 3, 7-trimethyl xanthine) activates the central nervous system. Consuming a high amount of caffeine may cause damage to the liver but some earlier studies conducted by Shan *et al.,* 2022 have shown that moderate coffee intake is beneficial for various liver diseases possibly by inhabiting adenosine binding by inhabiting adenosine binding to its receptors. Alnuimy, (2021) used different doses of caffeine on albino mice which were pregnant, the most important histopathological conditions observed were leisons, coagulative necrosis, nucleus pyknosis, dilated sinasoids, hypertrophy and degenerated vacuolated and apoptosis in both pregnant mice and fetal liver. In the present study no bacteria was detected from blood on nutrient agar or blood agar.

Kerrigan and Lindsey, (2005) suggested that abnormally high dose of caffeine may result in death. The femoral blood of a female with history of intravenous drug intake containing 192 mg/L caffeine, while in the other case a 29 years old male with history of diabetes and obesity contained 567 mg/L caffeine. Aknouche *et al.,* (2017) in a case history recorded death of man aged 48 who had used 40/mg/L which may corresponds to a fatal concentration. Almosawi *el al.,* (2018) suggested that anxiety was observed in treated with moderate as well as high doses of caffeine in the mice.

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