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"MACROSCOPIC AND MICROSCOPIC ALTERATIONS OF LIVER IN MALE ALBINO RATS INDUCED BY NICOTINE ADMINISTRATION; AN EXPERIMENTAL STUDY"

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Abstract

Introduction: Nicotine is the principal alkaloid contained in tobacco and is produced by plants of nightshade family. Nicotine has relatively high toxicity with varied effects on our body. The liver of albino rats resembles human liver both microscopically as well as microscopically. **Aims and objectives:** The aim of study was to study gross and histopathological changes induced by administration of graded doses of nicotine hydrogen tartrate in albino rats over a period. **Material & methods:** A total of 36 male albino rats were included in our study and after a period of acclimatization, were divided in to three groups of 12 rats each with one group serving as control while as other two groups received nicotine in concentration of 5mg/10ml and 7.5mg/10ml.The rats were sacrificed later to study for changes in liver anatomy. **Results and observation:** The most common changes were progressive decrease in weight of albino rats and pentad of microscopic changes including central venous congestion, portal venous congestion, sinusoidal congestion, fatty changes of hepatocytes and inflammation of portal triads. **Conclusion:** The findings of our study clearly revealed that changes induced in microanatomy of liver in male albino rats were directly related with dosage and duration of nicotine administration and considering the similarity between human liver and liver of male albino rats, the therapeutic administration of nicotine in human population should be rigorously studied by more experimental studies.

Key words: Nicotine, Liver, microscopic changes

Introduction: Nicotine, a nitrogen-containing chemical, is the principal alkaloid contained in tobacco. It is believed to be the primary reason for cigarette smoking for many people, as they derive satisfaction and a pleasant sensation from inhaling nicotine. Nicotine is the main component of tobacco smoke, and failure to quit smoking is virtually attributed to its addictive potential, which is like that of opium and alcohol. It is produced in the roots of the plant, accumulates in the leaves, and is also found in smaller amounts in other members of the Solanaceae family [4, 6]. It is widely consumed through cigarette smoking and tobacco chewing by 30–40% of the world's population. Nicotine is readily volatile and dibasic. It is a hygroscopic, colourless to yellow-brown, oily liquid. An average tobacco rod contains 10–14 mg of nicotine, and on average, about 1–1.5 mg of nicotine is absorbed systemically during smoking.[8,9] Nicotine is naturally produced in the nightshade family of plants, mostly in leaves

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in the range of about 0.5 to 7.5%. It constitutes approximately 0.6–3% of the dry weight of tobacco and is present in the range of 2–7 μg/kg in various edible plants.[14.] Nicotine is also present in various foods, weeds, teas, etc. Common foods include tomatoes, potatoes, eggplant, cauliflower, green pepper, capsicum, and the leaves of the coco plant. Eggplants are the richest source of nicotine and contain nicotine at a concentration of 100 ng/g. The concentration is considerably higher in green tomatoes, which is reported at about 42.8 ng/g compared to 4.3 ng/g in ripe tomatoes. Nicotine has a relatively high toxicity in comparison to many other alkaloids, such as caffeine, which has an LD_{50} of 127 mg/kg when administered to mice.

Like human liver, rat liver is reddish brown in colour, irregular in shape, and smooth in appearance. It consists of four lobes, namely left, right, caudate, and middle, and has eight segments. The caudate and left lobes contain one segment each; the middle lobe has four segments, and the right lobe has two segments. In contrast to humans, albino rats, like ruminants, lack gallbladders. The function of the gallbladder, like the concentration of bile, is performed by cells lining the hepatobiliary duct system. Microscopic anatomy does not vary in humans or albino rats. Microscopically, the lobules are roughly hexagonal and consist of plates of hepatocytes radiating from a central vein that joins the hepatic vein to carry blood out of the liver. A distinctive component of a lobule is the portal triad, which can be found running along each corner of the lobule. The portal triad, misleadingly named, consists of five structures: a branch of the hepatic artery, a branch of the hepatic portal vein, and a bile duct, as well as lymphatic vessels and a branch of the vagus nerve. Between the plates are liver sinusoids, which are enlarged capillaries through which blood from the hepatic portal vein and hepatic artery enters via the portal triads and then drains to the central vein. These sinusoids were lined by discontinuous endothelium, and macrophages were seen in the sub-sinusoidal space.

Material Methods: The experimental study was conducted in the Postgraduate Department of Anatomy, Government Medical College Srinagar, after proper ethical clearance. The study was done to see the changes in weight and microanatomy of the liver in male albino rats after oral administration of graded doses of nicotine. Thirty-six male Albino rats weighing on average 150–200 grams were taken from the Animal House of Govt. Medical College Srinagar for this study. The chemical used in this experiment was nicotine hydrogen tartrate ($C_{18}H_{26}N_2O_{12}$). It was given as a colourless powder mixed in drinking water. Nicotine solutions with concentrations of 5 mg/ml and 7.5 mg/ml were prepared using tap water and nicotine powder.

Experiment Design: -The rats were allowed to acclimatize for three weeks in the animal house of the government medical college in Srinagar and were kept in separate cages. The animals were divided into two main groups: control (group 1) and study (group 2) groups. The study group was further divided into 2A and 2B groups.

1. **Group 1(Control group); Formed of 12 rats, received plain water, and were fed a normal diet.**
2. **Group 2(study group); This was divided in to two subgroups.**

a) **Group 2A;** is formed of 12 rats. I received 5 mg/10 ml of nicotine solution daily for 16 weeks in addition to my normal diet.

b) **Group 3B;** contains 12 rats. I received 7.5 mg/10 ml of nicotine solution daily for 16 weeks in addition to my normal diet.

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Specimen collection: At the assigned time, animals were sacrificed after anesthetizing the rats using formalin inhalation. In every session before scarifying the animals, the weight was recorded using an electronic weighing machine. The first three sittings were done with a duration of four weeks between each sitting, and the last sitting was done after stopping the drug for four weeks. In each sitting, rats were sacrificed after anesthetizing them with chloroform. The limbs of rats were fixed on board with pins, and a midline abdominal incision was made. Organs were identified, dissected, cleaned, and after examining for gross changes were put in dishes containing formaldehyde. These tissues were processed manually for block-making using standard histological techniques. Sections measuring 5–6 micrometres were cut and fixed on glass slides.

Observation and Results: Weight: There was a progressive increase in weight in group 1 (control) from the first week, and comparatively, there was a progressive decrease in weight in both the 2B (high dose) and 2A (low dose) groups. However, in the recovery period, once the drug was stopped for 4 weeks, there was a gradual increase in weight in both the 2B (high dose) and 2A (low dose) groups.

Table a: Mean ± SD weight of rats of three groups recorded at different weeks of study:

	Weight in grams (mean ± SD)		
	Control (1)	**Low Dose(2A)**	**High Dose(2B)**
Week 0	162.5 ± 12.9	152.1 ± 4.0	163.3 ± 12.3
Week 4	172.9 ± 12.5	146.3 ± 4.8	153.3 ± 12.3
Week 8	189.4 ± 10.1	142.2 ± 5.1	146.7 ± 12.2
Week 12	210.8 ± 10.2	139.2 ± 4.9	135.0 ± 13.8
Week 16	231.7 ± 10.4	148.3 ± 5.8	156.7 ± 5.8

Table b:

Comparison of the weight of rats at weeks 4, 8, 12, and 16 with the weight at week 0 in three groups using the Wilcoxon Signed Ranks Test.

Comparison weeks	**p-value**		
	Control (1)	**Low Dose(2A)**	**High Dose(2B)**
Week 0–Week 4	0.001	0.001	0.001
Week 0–Week 8	0.006	0.004	0.003
Week 0–Week 12	0.026	0.014	0.014
Week 0–Week 16	0.109	0.083	0.083

Liver: On gross examination, there seems to be no apparent change in the liver at any point in time after drug administration, but we have observed changes at the microscopic level.

Microscopic changes:

1. Central venous congestion: There was no central vein congestion in Group 1 (control). The low-dose group showed mild changes at week 4 and moderate at week 8, while the high-dose group showed mild changes at week 4, moderate at week 8, and severe congestion at week 12 (Fig. 1). There was complete recovery once the drug was stopped in the low-dose group and partial recovery in the high-dose group.

Table 1a: Distribution of severity of central vein congestion in the liver of the three groups.

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`Group	Central vein congestion in the liver			
	Absent	**Mild**	**Moderate**	**Severe**
Control (1)	12 100.0%	0 0.0%	0 0.0%	0 0.0%
Low Dose (2A)	3 25.0%	3 25.0%	6 50.0%	0 0.0%
High Dose (2B)	0 0.0%	6 50.0%	3 25.0%	3 25.0%

Table 1b: Comparison of the severity of central vein congestion in the liver of the three groups using the Kruskal-Wallis test.

	No.	**Mean Rank**	**p-value**
Control (1)	12	8.00	
Low Dose (2A)	12	21.50	<0.001
High Dose (2B)	12	26.00	

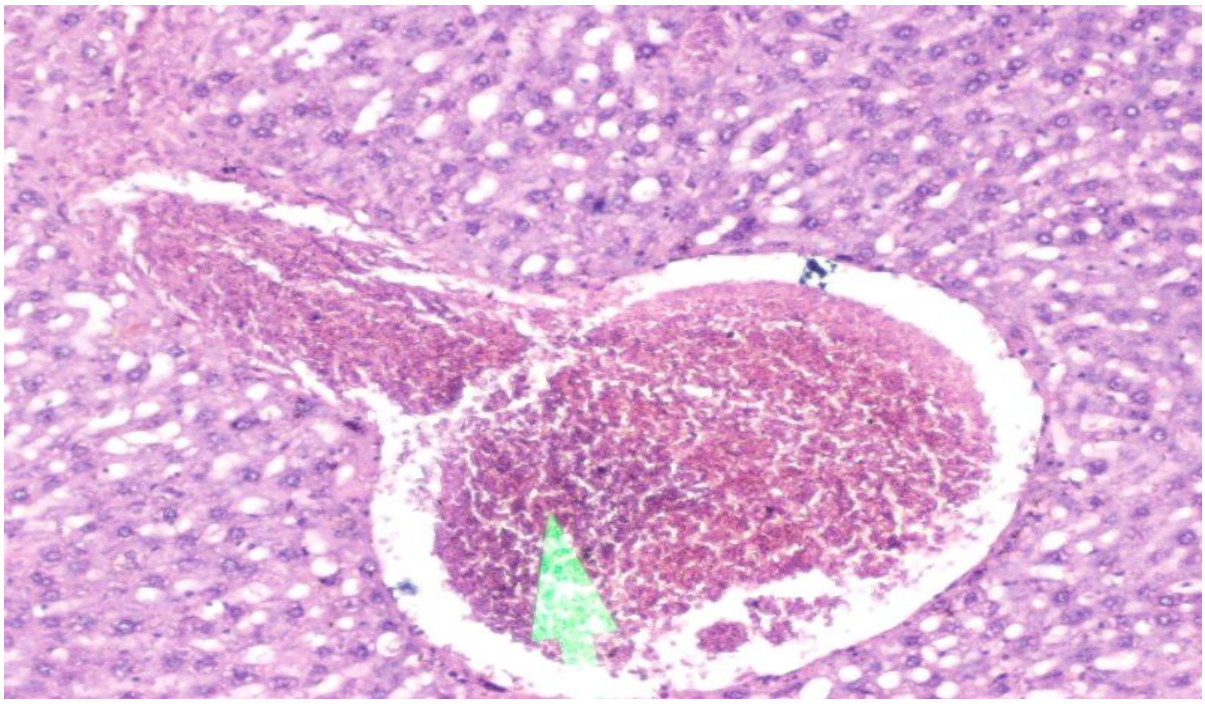

Fig. 1: Microphotograph of the liver of the 2B (high dose) group at week 8 after drug administration, showing central venous congestion (10x).

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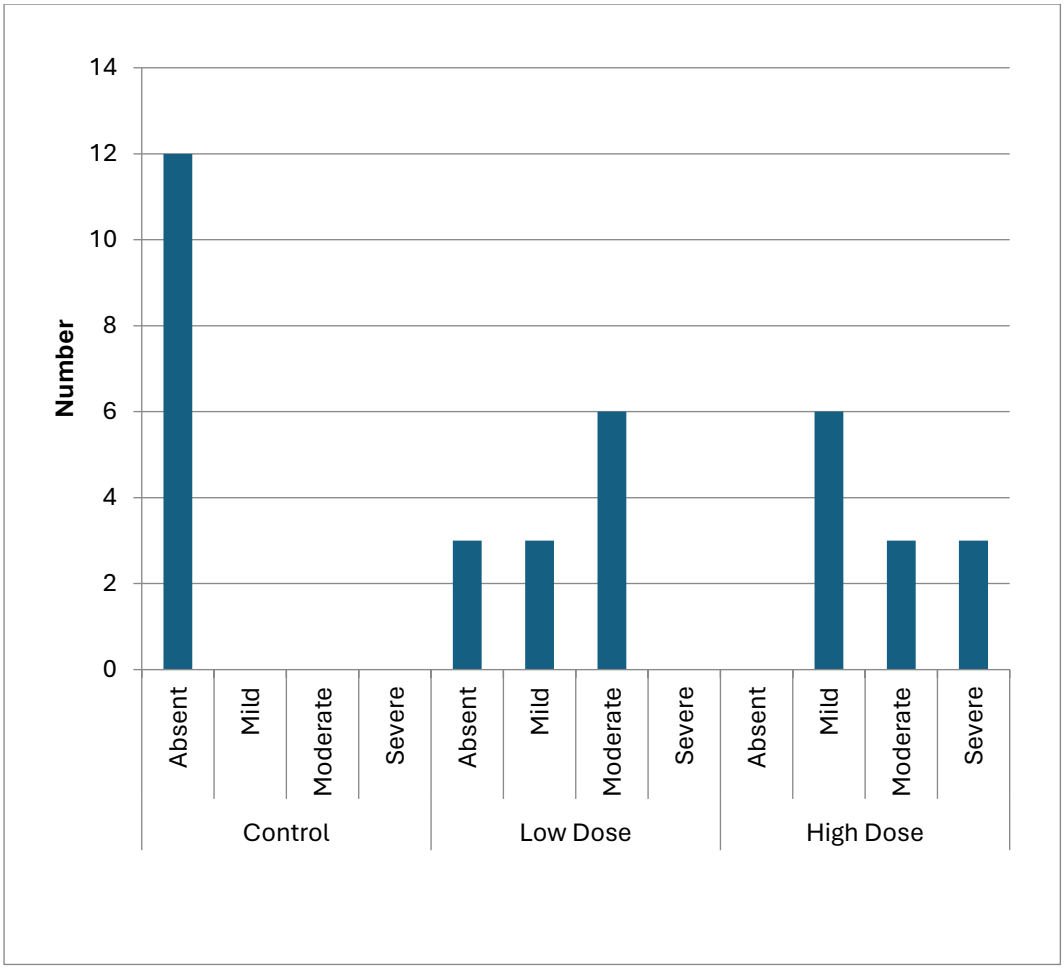

Figure 1: Distribution of severity of Central vein congestion in liver of the three groups

2. Portal venous congestion: Portal venous congestion was absent in the 1 (control) group. In both study groups, it appeared in the 8th week and was mild to moderate in both study groups (Fig. 2). There was complete recovery once the drug was stopped for 4 weeks.

Table 2a: Distribution of severity of portal vein congestion in the liver of the three groups.

	Portal vein congestion in the liver		
	Absent	**Mild**	**Moderate**
Control (1)	12	0	0
	100.0%	0.0%	0.0%
Low Dose (2A)	6	3	3
	50.0%	25.0%	25.0%
High Dose (2B)	6	3	3
	50.0%	25.0%	25.0%

Table 2b: Comparison of severity of portal vein congestion in the liver of the three groups using the Kruskal-Wallis test.

	No.	**Mean Rank**	**p-value**
Control (1)	12	12.50	0.015

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Low Dose (2A)	12	21.50
High Dose (2B)	12	21.50

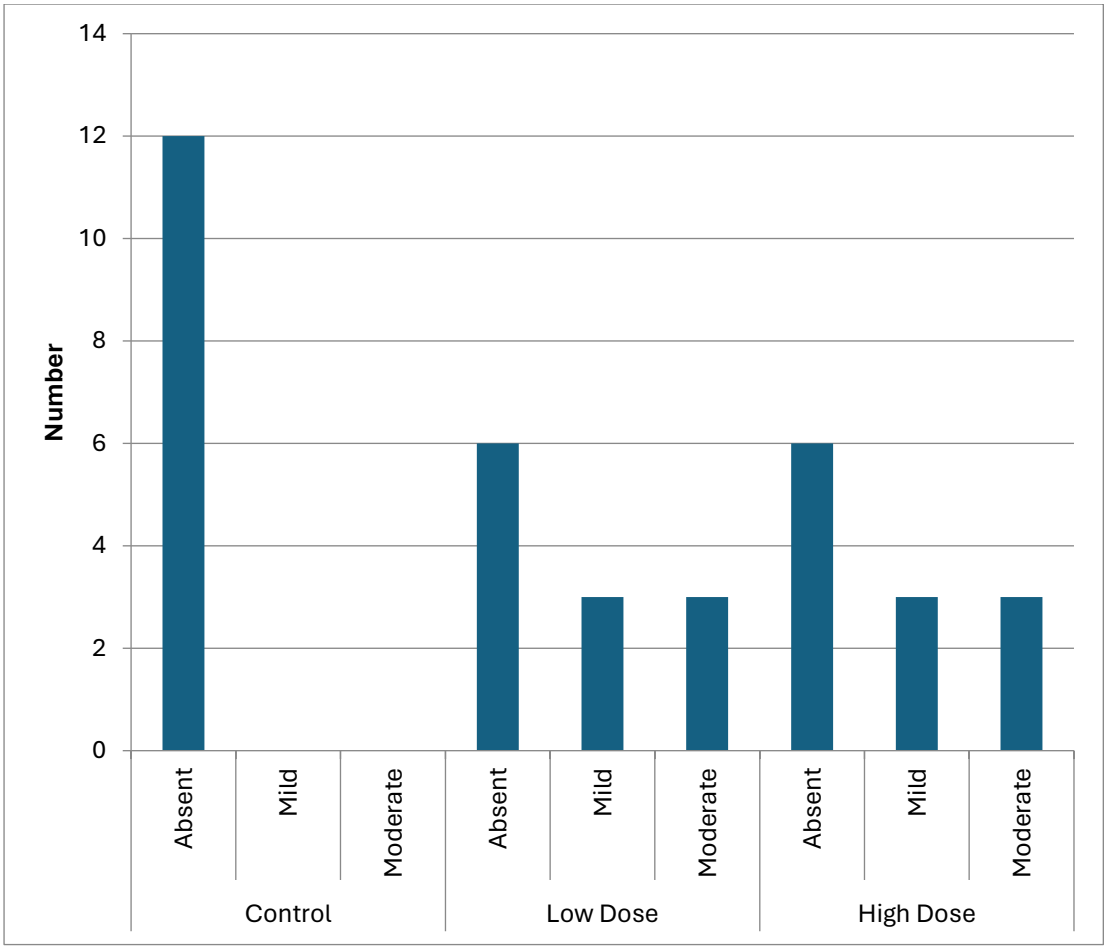

Figure 2: Distribution of the severity of portal vein congestion in the liver of the three groups

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Fig 2 microphotograph of liver of high dose group showing portal venous congestion at 20x.

3. Sinusoidal Congestion: -Sinusoidal congestion was absent in the control group; it appeared at 8 weeks in the low-dose group and was mild. In the high-dose group, it was mild at week 8 and moderate at week 12 (figs. 6 and 7). There was complete recovery in the low-dose group and partial recovery in the high-dose group once the drug was stopped for 4 weeks.

Table 3a: Distribution of severity of sinusoidal congestion in the liver of the three groups.

	Sinusoidal congestion in the liver		
	Absent	**Mild**	**Moderate**
Control (1)	12	0	0
	100.0%	0.0%	0.0%
Low Dose (2A)	6	6	0
	50.0%	50.0%	0.0%
High Dose (2B)	3	6	3
	25.0%	50.0%	25.0%

Table 3b: Comparison of the severity of sinusoidal congestion in the liver of the three groups using the Kruskal-Wallis test.

	No.	**Mean Rank**	**p-value**
Control (1)	12	11.00	
Low Dose (2A)	12	19.25	0.001
High Dose (2B)	12	25.25	

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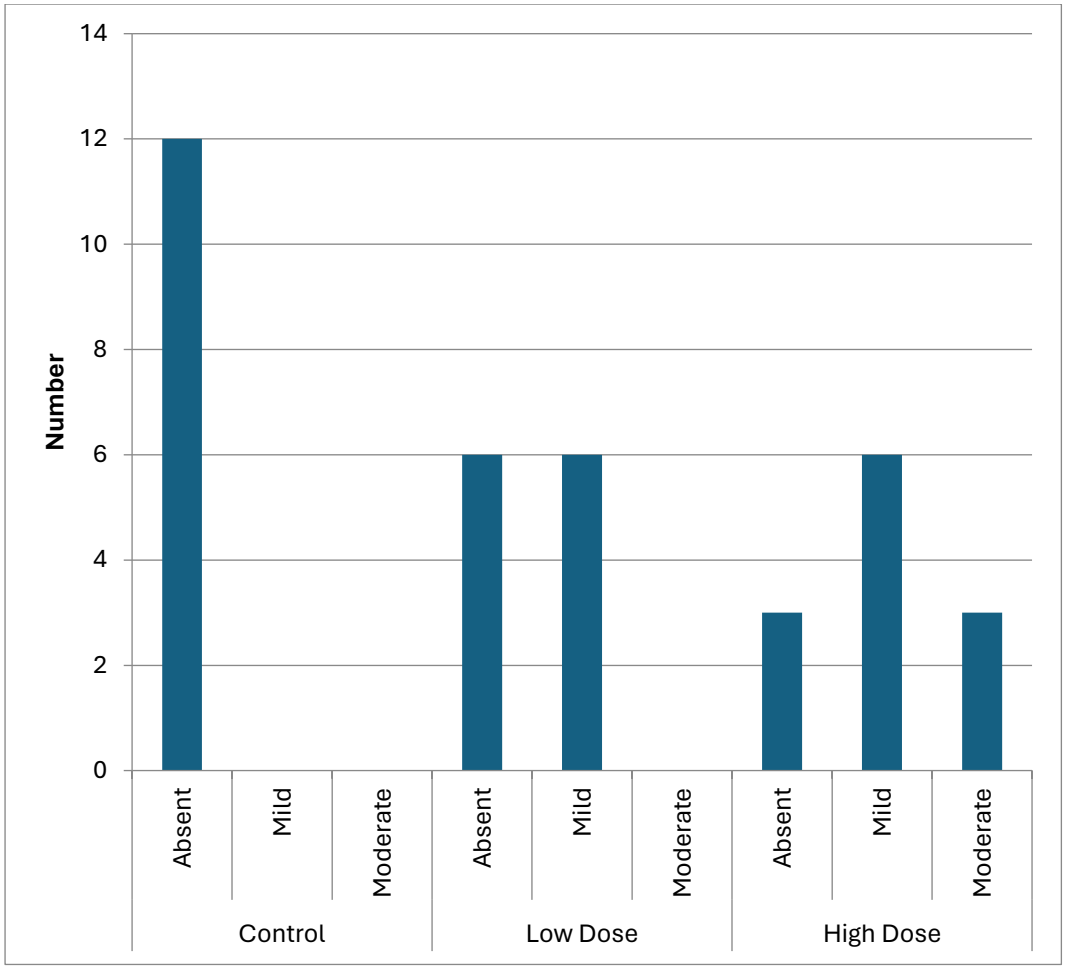

Figure 4: Distribution of the severity of sinusoidal congestion in the liver of the three groups

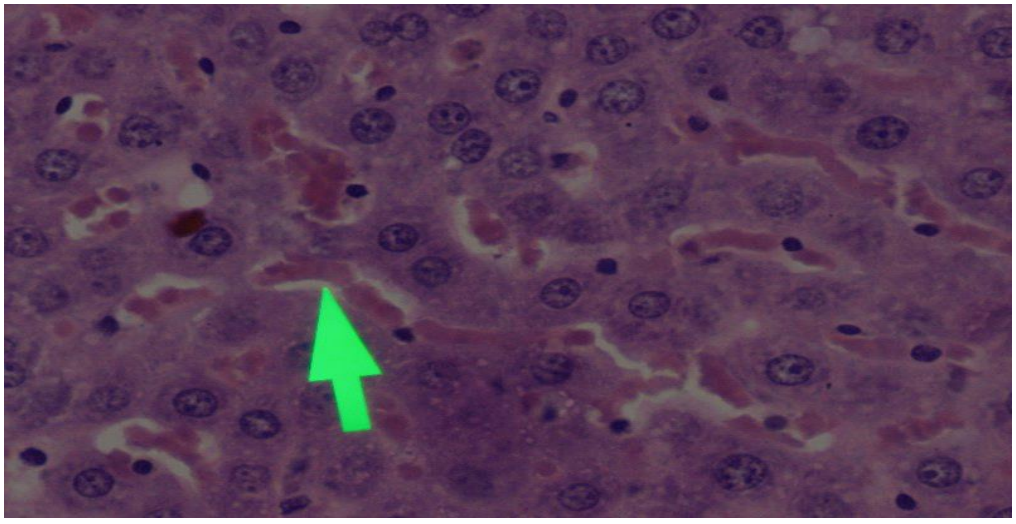

Fig 3; microphotograph of liver at 12 week showing sinusoidal congestion at 40x

3. **Fatty changes**: Fatty changes were absent in the control group, appeared at week 12 in the low-dose group, and were mild. In the case of the high-dose group, it was mild at week 8 and moderate at week 12 (figs. 4 and 5). There was complete recovery during the recovery period in the low-dose group and partial recovery in the high-dose group.

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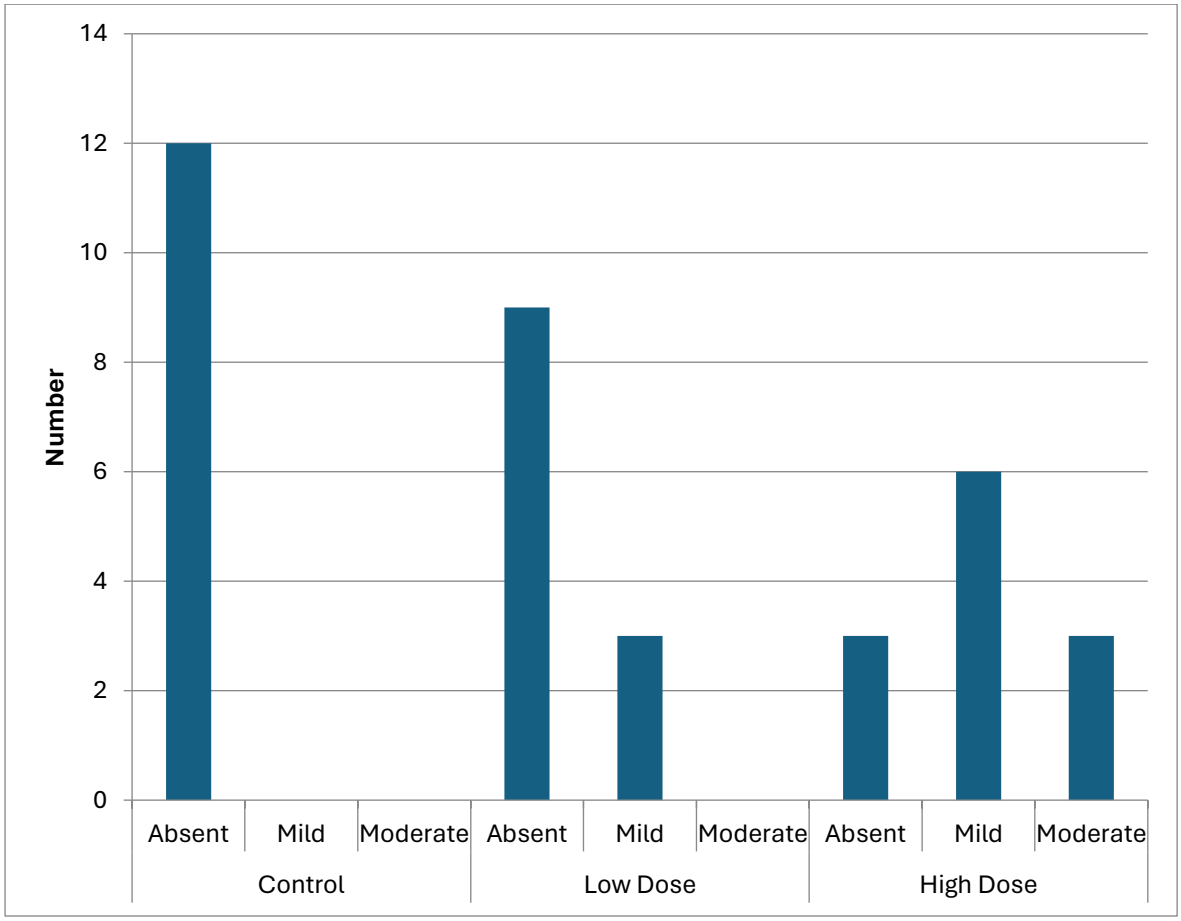
Table 4a: Distribution of severity of fatty changes in the liver of the three groups.

	Fatty changes in the liver		
	Absent	**Mild**	**Moderate**
Control (1)	12 100.0%	0 0.0%	0 0.0%
Low Dose (2A)	9 75.0%	3 25.0%	0 0.0%
High Dose (2B)	3 25.0%	6 50.0%	3 25.0%

Table 4b: Comparison of the severity of fatty changes in the liver of the three groups using the Kruskal-Wallis test.

	No.	**Mean Rank**	**p-value**
Control (1)	12	12.50	
Low Dose (2A)	12	16.63	<0.001
High Dose (2B)	12	26.38	

Figure 5: Distribution of the severity of fatty changes in the liver of the three groups



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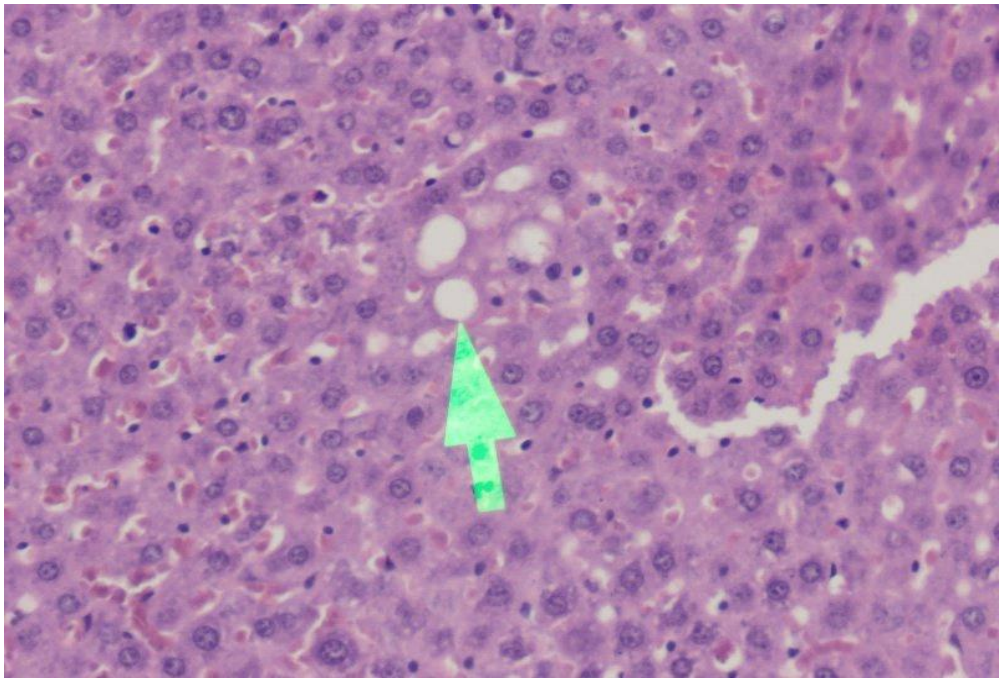

Fig 4; microphotograph of liver showing fatty changes at 40x

5. Inflammatory changes: -Inflammatory changes were not seen in the control group and were mild at week 12 in the case of the low-dose group. In the case of the high-dose group, it appeared at week 8 and was mild to moderate (figs. 3 and 8). During the recovery phase, there was a partial recovery in the high-dose group and a complete recovery in the low-dose group.

Table 5a: Distribution of severity of inflammatory changes in the liver of the three groups.

	Inflammatory changes in the liver		
	Absent	**Mild**	**Moderate**
Control (1)	12	0	0
	100.0%	0.0%	0.0%
Low Dose (2A)	9	3	0
	75.0%	25.0%	0.0%
High Dose (2B)	3	6	3
	25.0%	50.0%	25.0%

Table 5b: Comparison of the severity of inflammatory changes in the liver of the three groups using the Kruskal-Walli's test.

	No.	**Mean Rank**	**p value**
Control (1)	12	12.50	
Low Dose (2A)	12	16.63	<0.001
High Dose (2B)	12	26.38	

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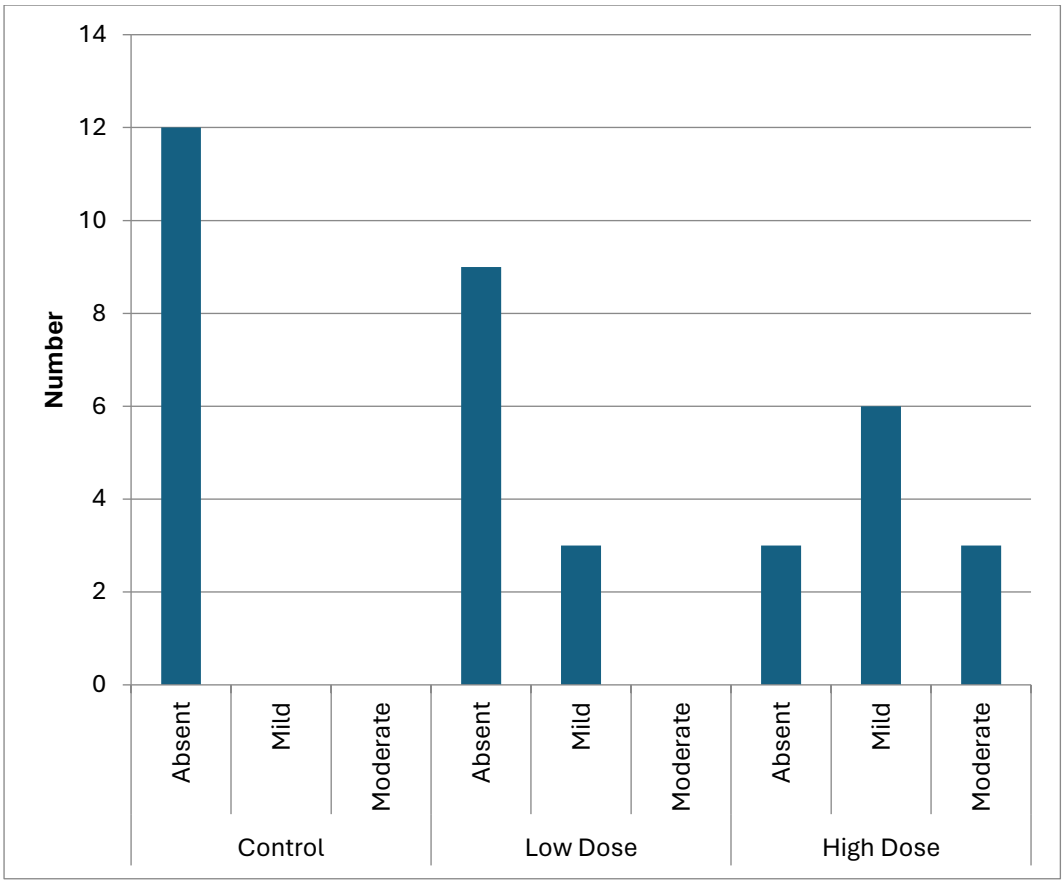
Figure 6: Distribution of severity of inflammatory changes in the liver of the three groups.



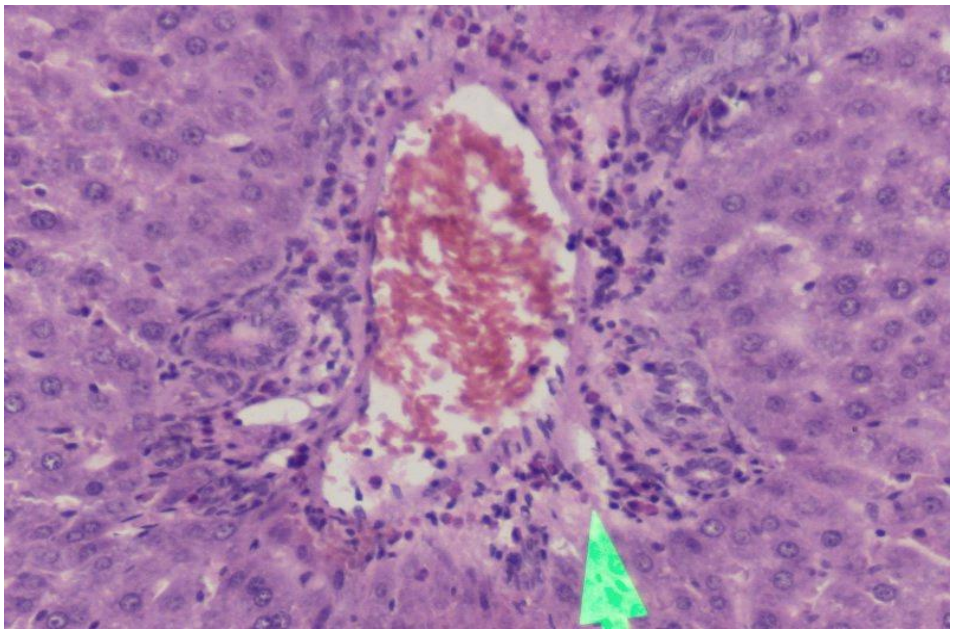


Fig 5; microphotograph of liver showing inflammatory changes around portal triad at 40x.

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Discussion: Nicotine dependence results in substantial mortality, morbidity, and socioeconomic impacts. [7] Moreover, its dependence is a serious public health concern, as it is one of the leading causes of avoidable deaths worldwide. Studies have shown that the liver shows deposition of adipose tissues in portal veins, hepatomegaly, and lipid accumulation in cells because of a disease of parenchyma caused by nicotine intake.[5,4] Degenerative changes in hepatocytes, cellular infiltration, periportal fibrosis, and congestion of both the central and portal veins have been noticed. Nicotine is classified as a poison. [6,7] However, at the controlled dosage used by consumers, its effects are minimal.

WEIGHT: During the study, there was a progressive increase in the weight of rats in the control group. As in Study Groups (2A and 2B), once the drug was administered, there was a decrease in weight for both the high-dose (2A) and low-dose (2B) groups. At the 16th week, once the drug was stopped for 4 weeks, there was an increase in weight in both high-dose and low-dose groups. In our study, the results conformed with the results of **Mamdouh A. Ghaly et al. (200354),** while working on a comparative study of the effects of nicotine on the liver of albino rats. In their study, weight records showed that the rats who inhaled cigarette smoke and those who were injected with nicotine revealed a significant decrease in body weight. However, during the recovery period, a progressive increase in body weight was seen.

LIVER: As far as the liver is concerned, there were no apparent macroscopic or microscopic changes in Group 1 (control). During the detailed examination, the liver was normal in study groups as well; however, there were microscopic changes seen in the form of central venous congestion, portal venous congestion, sinusoidal congestion, fatty changes, and inflammatory infiltration of mononuclear cells around portal triads in study groups. Central venous congestion appeared at week 4 and was mild, moderate at week 8, and more marked at week 12 in the 2A group. In the case of the 2B group, CVC appeared at week 4 and was mild; later, it became moderate at week 8 and severe at week 12. Portal venous congestion was seen at week 8 and was mild at week 8 and moderate at week 12 in both the low-dose group and the high-dose group. Sinusoidal congestion was seen at week 8 and was mild in the low-dose group. In the case of the 2B group, it was mild at week 8 and moderate at week 12. A focal cellular inflammatory infiltrate was seen around portal triads at week 12 in the 2A (low dose) group. In the case of the 2B (high dose) group, it was mild at week 8 and moderate at week 12. Fatty changes were seen in week 12 and were mild in the 2A group, while in the case of the 2B group, they were mild at week 8 and moderate at week 12. These changes were more marked in the 2B (high dose) group as compared to the 2A (low dose) group. While studying, it was noticed that these changes were not widely distributed all over the whole liver, and some focal areas of the liver were still attaining normal structure. Complete recovery of portal venous congestion was seen in the 2B (high dose) group after stopping the drug for 4 weeks, while Partial recovery of central venous congestion, sinusoidal congestion, cellular inflammatory infiltration, and fatty changes were seen in the 2B (high dose) group after stopping the drug for 4 weeks. Complete recovery of central venous congestion, portal venous congestion, sinusoidal congestion, cellular inflammatory infiltration, and fatty changes were seen in the 2A (low dose) group after stopping the drug for 4 weeks. **Mamdouh A. Ghaly et al. (2003)** had almost similar results while working on a comparative study of the effects of nicotine on the liver of albino rats used via two routes: inhalational and subcutaneous. Their finding was more intense in comparison to our study, possibly because of the use of cigarette smoke containing many

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chemicals in the inhalational group and the higher systemic concentration of nicotine in the subcutaneous group, unlike our study where pure nicotine in oral form was used. **Samuel Santos Valencia et al. (2008)** had similar results while working on the effects of oral nicotine on rat liver histology; however, in our study, we noticed more sinusoidal, central vein, and portal vein congestion, possibly because of the longer duration and higher doses.

Conclusion: The present study was undertaken in the postgraduate department of Anatomy Government Medical College Srinagar. The objective of this study was to evaluate changes in weight and the microanatomy of the liver in male albino rats treated with oral nicotine. A total of 36 male albino rats were taken and were divided into two groups. The control group contained 12 rats, and the study group contained 24 rats. The study group was further divided into two groups: the 2B (high dose) group and the 2A (low dose) group, each containing 12 rats. Three rats from each group were sacrificed at the 4th, 8th, 12th, and 16^{th} weeks at a regular interval of 4 weeks. In the livers of rats, it was found that the rats treated with nicotine showed sinusoidal congestion, central venous congestion, portal venous congestion, fatty changes, and inflammatory changes. These findings were more profound in the high-dose group and increased progressively with time, but once the drug was stopped, there was a partial or complete recovery of these changes. However, there was no apparent change during the macro-examination. Weight increased progressively in the control group throughout the study, but in the cases of the high-dose group and the low-dose group, there was a decrease in weight once the drug was administered. At week 16, there was an increase in weight in both high-dose and low-dose groups as the drug was stopped for 4 weeks. As already discussed, the findings were less intense as compared to the other studies, possibly because of the dosage, duration, and method of administration of the nicotine.

In summary, our study revealed a multitude of effects on liver and weight in male albino rats. Considering the marked anatomical similarity between human and rat anatomy, nicotine may have similar effects on human body organs, thus one must be extra conscious while prescribing nicotine as a drug in modern medicine besides shutting doors of all ways of abusing nicotine.

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