

ORIGINAL ARTICLE

The Protective Effect of Olive Oil on Arsenic Induced Histological Changes in the Liver of Albino Rats

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ABSTRACT

Objective: To study the protective effect of olive oil against histological changes induced by arsenic in liver of albino rats.

Study Design: Randomized control trail.

Place and Duration of Study: The research was carried out from 1st to 30th November 2017 at National institute of Health, Islamabad.

Materials and Methods: Forty five male adult albino rats were placed in three cages having 15 rats each. Distilled water was given to rats of control group I for 30 days. The rats of group II were given 40mg per kg per day of Sodium Arsenite dissolved in drinking water for 30 days. Rats in group III, in addition to sodium Arsenite received olive oil, 0.2ml per day for 30 days along with Sodium Arsenite. Dissection was done after 30 days and liver was dissected out for histological changes.

Results: The use of olive oil improved the gross and microscopic changes induced by Arsenic in liver lobes(right lateral and left lateral) of Albino rats of group III as compared to group II rats, which received only arsenic. Among microscopic parameters, sinusoidal dilation, pyknosis and necrosis was markedly reduced by use of olive oil in group III rats whereas hemorrhage was absent in group III.

Conclusion: Olive oil protects histological changes caused by arsenic in liver of Albino rats which include sinusoidal dilation, congestion, pyknosis, necrosis and haemorrhage.

Key Words: *EVOO (extra virgin olive oil), Sodium arsenite, Oleuropein.*

Introduction

Liver, the largest organ in the body, usually weighs about 1.5 kg. It is an organ of metabolism and production of energy; its other main functions include: storage of iron, trace elements, vitamins and bile production. The weight of human liver is 2 to 3%, whereas rat liver is 5% of total body weight.¹ Various metals have acute and chronic effects on liver; Arsenic is one of them which also produce toxic effects on liver. Arsenic, a “protoplasmic poison” interferes with mitosis, cell respiration, enzymes due to its effect on sulfhydryl group of cells.² It can also exert its toxic effects by generating “reactive oxygen

species (ROS)” and “reactive nitrogen species (RNS)” leading to necrosis, oxidative damage to proteins, lipids and DNA in cells.^{1,3,4} Several acute and chronic hepatic effects have been associated with arsenic poisoning.⁵ Various antioxidants are available which can reduce the effect of arsenic on liver and various organs, olive oil is one of them which can be used to avoid disastrous effects of arsenic on liver. Olive oil contains about 70% Oleic acid and phenolic compounds that provide health benefits. Olive oil itself has a greater antioxidant capacity than most other seed oils.⁶

Arsenic affects most of the organs involved in absorption, accumulation and excretion,⁷ Long-term exposure to inorganic arsenic can cause dysfunction of endocrine system^{8,9} nervous system,¹⁰ and reproductive system,¹¹ and may also cause loss of body weight.⁴ Exposure to arsenic also causes, liver fibrosis, metabolic disorder such as diabetes,^{12,13} chronic lung disease, gangrene of toes,⁹ cancer of internal sites and skin.¹⁴ It has been found to have toxic effects on gonadal tissues of laboratory animals as well.¹¹

Various antioxidants have been used to ameliorate

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toxic effects of arsenic on various organs. Vitamin E, Ca and Olive oil is used to improve Arsenic induced histological changes in ovary.^{15,6} Antioxidants like L ascorbic acid, biochanin A, menthe piperita and aloe Vera has been used to improve toxic effects of arsenic on liver of rats.^{16,19} Oleuropein, one of the component of olive oil is found to be effective antioxidant in literature, therefore olive oil may be used in our study to ameliorate arsenic induced hepatotoxic effects.²⁰ The present study was designed to study the protective effect of olive oil against histological changes induced by arsenic in liver of albino rats.

Materials and Methods

The experiment was carried on the basis of randomized control trial under supervision of animal house at NIH Islamabad from 1st to 30th November 2017. Forty five male albino rats, weighing 250 to 300gm were kept in three cages with a number of 15 rats per cage. The simple random sampling technique was used. The research was approved by Ethical Review Committee. A controlled standard living environment suitable to their class with adjusted diet was given. A well ventilated room with cycles of 12 hours light and 12 hours dark were maintained under 20 to 26 °C. The rats were adult of age 2 to 4 months and those with any known pathology and female rats were excluded. Animals were grouped accordingly as mentioned below.

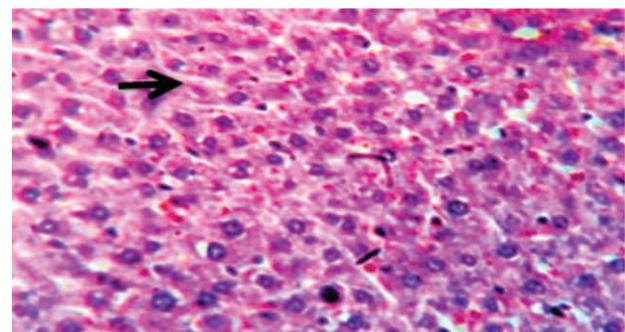
Group I Control group n = 15	Control group rats consumed distilled water as drinking water for 4 weeks. These rats were dissected after 4 weeks.
Group II Experimental group n= 15	Rats consumed a solution of arsenic (40mg/kg) as sodium arsenite for 4 weeks They were also dissected and observed after 4 weeks to observe any change in histology of liver lobes.The longitudinal sections were taken from left lateral and right laterateral lobes.
Group III Experimental group n = 15	Rats consumed a solution of arsenic (40mg/kg) as sodium arsenite along with olive oil 0.2ml/day for 4 weeks. After 4 weeks these rats were analyzed to observe hepatotoxic effects of arsenic which were prevented due to use of olive oil.

After accomplishment of 4 weeks duration of experiment, rats were anesthetized with chloroform and dissected. After fixation and embedding, eosin and hematoxylin stains were used for histological

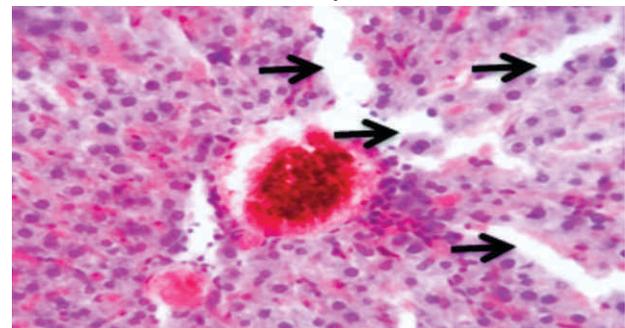
sections. The slides were examined in detail under X10 and X40 power of light microscope. The microscopic qualitative parameters were observed which include Sinusoidal dilation, Congestion, Pyknosis, Necrosis and haemorrhage

Results

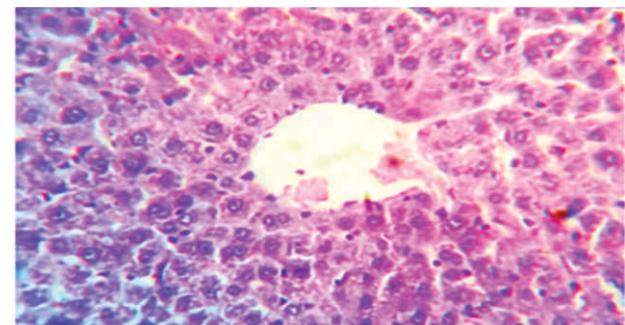
In control group I, experimental animals showed normal sinusoids while in group II and group III sinusoidal dilation was present in 100% of experimental animals. The use of olive oil in group III has significantly reduced the severity and amount of sinusoidal dilation caused by arsenic Table 1 and Figure 1).



Group I



Group II



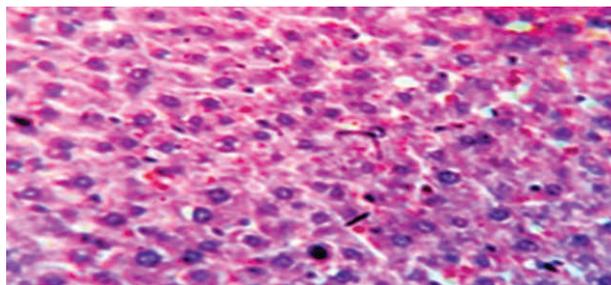
Group III (mild sinusoidal dilation)

Fig 1: Group Wise Distribution of Sinusoidal Dilation of Hepatic Lobule Showing Normal Sinusoid in Group I L7B1, Severe Sinusoidal Dilation in Group II L4D and Mild Sinusoidal Dilation in Group III L6 A.(H and E,X 40) (Indicated by Arrows in Group 1 and Group II of Figure 1)

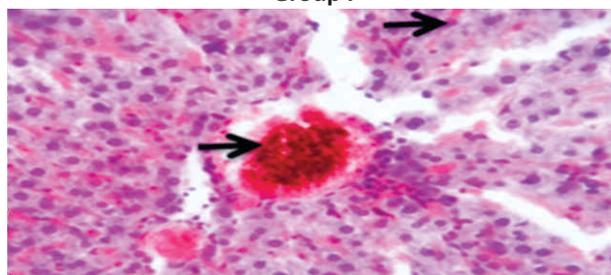
Table I :Group Wise Distribution of Sinusoidal Dilatation in Hepatic Lobule Among Control and Experiental Groups of Albino Rats

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	15(100%)	0(0%)	15	
Total	30	15	45	

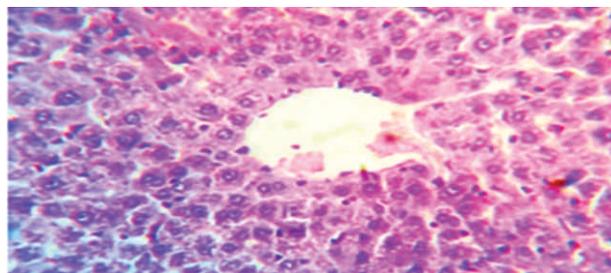
The control group showed no congestion in sinusoids and central vein. 100 % of experimental animals in group II showed congestion mainly in central vein and also in sinusoids, whereas 46.7% of rats in group III showed congestion but 53.3% showed no congestion. In this way olive oil has decreased the number of rats in group III showing congestion. (figure 2)



Group I



Group II Congestion in Central Vein and Sinusoids



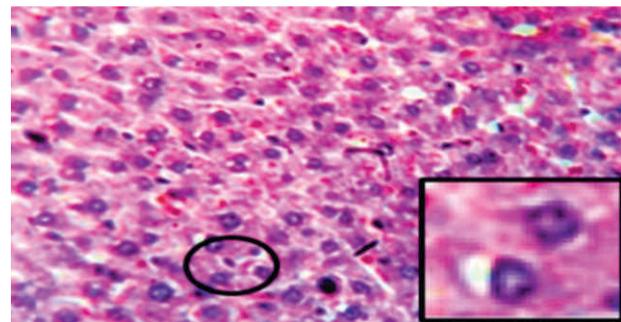
Group III

Fig 2: Group Wise Distribution of Congestion of Sinusoids and Central Vein Showing Normal Sinusoids and Central Vein in Group I L7D, Congestion in Central Vein and Sinusoid in Group II L4B, Whereas no Congestion in Group III L3C. (H and E, X 40). (Indicated by Arrow Heads in Group II Figure 2)

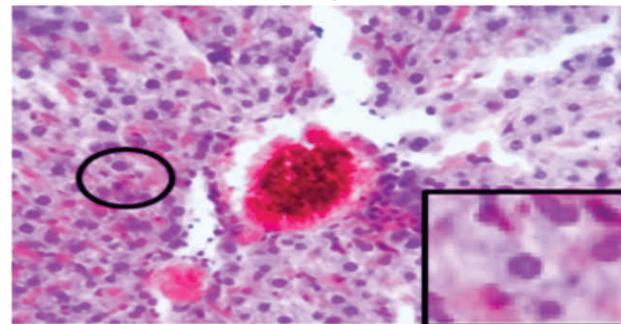
Table II: Group Wise Distribution of Congestion of Sinusoids and Central Vein Among Control and Experimental Groups

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	7(46.7%)	8(53.3%)	15	
Total	30	15	45	

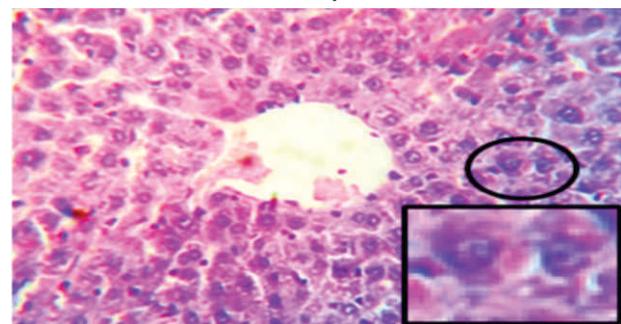
There was normal size of nucleus of hepatocytes in the control group I, pyknosis was present in 100 % of experimental animals of group II, Olive oil in group III has significantly reduced the number of rats showing pyknosis to 60%. (Table 4) (figure 3).



Group I



Group II



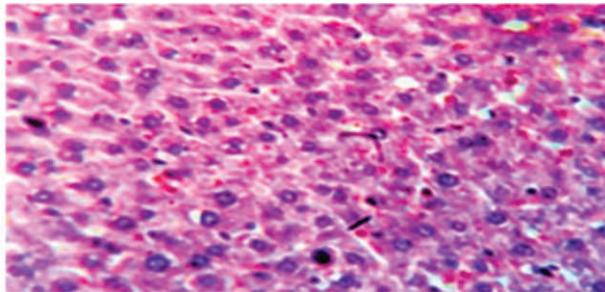
Group III

Fig 3: Group Wise Distribution of Pyknosis of Hepatocytes Among Control and Experimental Groups Shows Normal Nuclear Size in Group IL7A, Presence of Pyknosis in GroupII L4D and Absence of Pyknosis in Group III L3 B(H and E,X 40) .(Indicated by Circles in Figure 3)

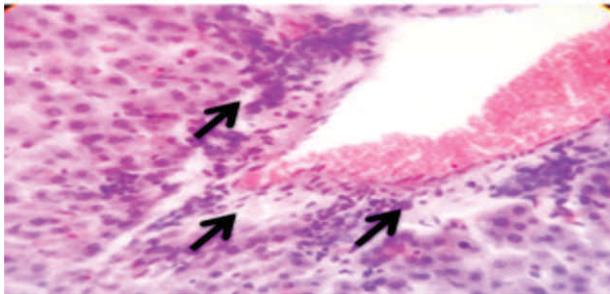
Table III: Group Wise Distribution of Pyknosis of Hepatocytes among Control and Experimental Groups of Albino Rats

Groups	Yes N (%)	No N (%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	9(60%)	6(40%)	15	
Total	24(53.3%)	21(46.7%)	45	

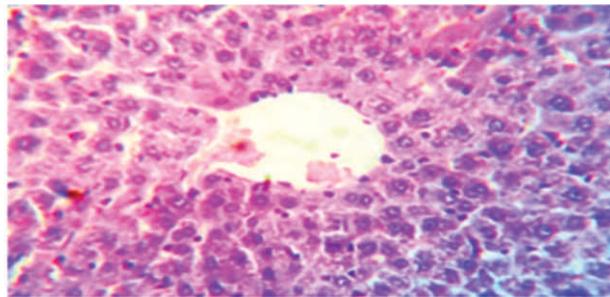
The normal hepatic parenchyma was observed in group I while 100% of experimental rats in group II showed necrosis in hepatic parenchyma, whereas use of olive oil along with arsenic in group III has significantly reduced the necrosis to 53.3% of rats in group III



Group I

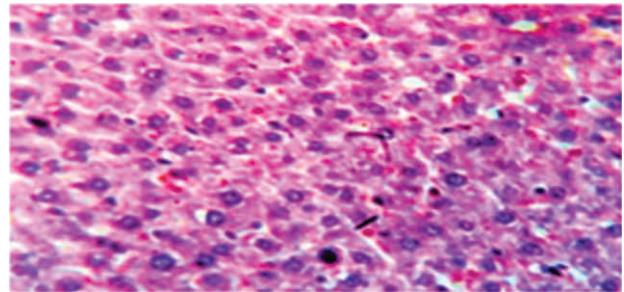
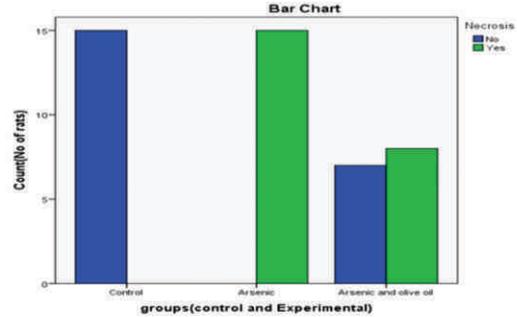


Group II (periportal necrosis)

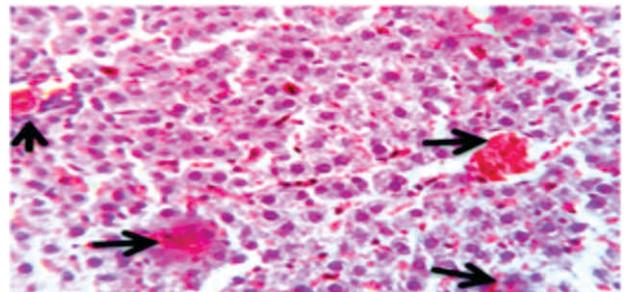


Group III

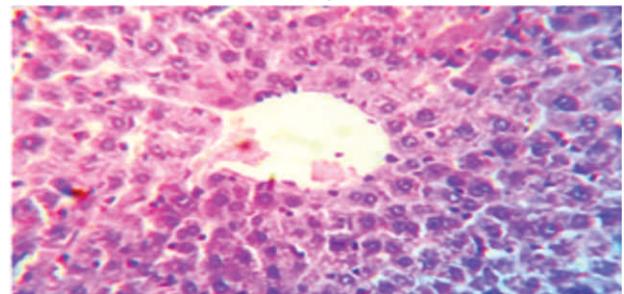
Fig 4: Group Wise Distribution of Necrosis of Hepatic Parenchyma among Rat Groups Shows Normal Hepatic Parenchyma in Group I L7A2, Presence of Coagulative Necrosis in Periportal Areas in Group II L5B, and Absence of Necrosis in Group III L3A(H and E,X 40). (Indicated by Arrow Head in Group II Figure 4)



Group I



Group II



Group III

Fig 5: Bar Chart Showing Distribution of Necrosis of Hepatic Parenchyma among Rat Groups

In group I, hepatic parenchyma appeared to be normal, 46% of rats in group II showed hemorrhage. In group III, olive oil has significantly improved hemorrhage as it was absent in all rats. (Table IV)(Figure 6)

In group I, hepatic parenchyma appeared to be normal, 46% of rats in group II showed hemorrhage group III, olive oil has significantly improved hemorrhage as it was absent in all rats.(Table

IV)(figure6)

Figure 6 Group wise distribution of haemorrhage in hepatic parenchyma among groups shows normal parenchyma in group I L7 C, haemorrhage indicated by arrow in group II L4D whereas no haemorrhage in group III L6A. (H and E, X 40).

Table IV: Group Wise Distribution of Haemorrhage in Hepatic parenchyma among Control and Experimental Groups of Albino Rats

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	7(46.7%)	8(53.3%)	15	
Group III	0(0%)	15(100%)	15	
Total	7(15.6%)	38(84.4%)	45	

*P≤0.05

Discussion

The sinusoidal dilation was observed in groups I,II and III. In control group I, experimental animals showed normal sinusoids while in group II and group III sinusoidal dilation was present in 100% of experimental animals. The use of olive oil in group III has significantly reduced the sinusoidal dilation as compared to dilation in group III caused by arsenic. In one study carried out by Kharroub et al, sodium arsenite was given at 1mg and 10mg for 45 and 90 days respectively which showed that sinusoidal dilation is dose and duration dependent. The dilation was greater at 10mg of arsenic for 90 days as compared to 1mg at 45 days.²¹ In another study conducted by Sohini Singh, 4 to 10mg of arsenic was given for 30 days to rats which also caused sinusoidal dilation of liver.¹⁶ In group III, there was slight sinusoidal dilation which is due to hepatoprotective effect of olive oil given simultaneously with arsenic to this group. Farag in his study showed natural antioxidant effect to prevent sinusoidal dilation and congestion caused by oxidative damage to liver.²² Congestion of sinusoids and central vein is caused due to inflammation of liver. All rats of group II showed congestion mainly in central vein and also in sinusoids due to effect of arsenic. 46.7% of rats of group III showed congestion but 53.3% showed no congestion.²³ In the study conducted by Oyagbemi et al, arsenic was given to three different groups at different doses for 4 weeks, they showed congestion in hepatic vessels both in central vein and sinusoids.²⁴ The use of olive oil in group III has significantly

reduced congestion to only 46.7% of rats. In a study by Azab, when olive leaf extract was given along with carbendazim, then congestion in central vein and sinusoids was remarkably decreased as compared to the group which was given only carbendazim.²⁵

There was normal size of nucleus of hepatocytes in the control group I, pyknosis was present in 100 % of experimental animals of group II, Olive oil in group III has significantly reduced the number of rats showing pyknosis to 60%. In a study conducted by Somia Bashir of India, arsenic was given to three groups in three different doses for acute period of 24 hours and pyknosis was found only in group which was given higher dose. This study support that Pyknosis is early sign of necrosis.²⁶

Necrosis occurs as a result of inflammation of liver caused by arsenic and it occurs after sinusoidal dilation and congestion in which degeneration of hepatocytes takes place due to ischemia. In the recent study, there is absence of necrosis in experimental animals of group I while 100% of experimental rats in group II showed necrosis whereas 53.3% of rats in group III showed necrosis. In this way olive oil has significantly decreased necrosis. In group II, there was periportal necrosis which is zone I as well as zone III which is pericentral, least oxygenated. In group III treated by both arsenic and olive oil, there is only pericentral necrosis.²⁷ In one of the study carried out by Sujata Das, sodium arsenite was also given in dose of 40mg per kg in drinking water to mice for 30 days and he found periportal necrosis similar to recent study.²⁸ The use of olive oil in group III has improved necrosis in our study. Metin Ogun, used oleuropein in dose of 30mg per kg along with 5mg per kg of sodium arsenite for only 15 days and he found that group which was given only arsenic, 6 mice showed necrosis but the oleuropein group showed necrosis in only 1 mouse.

The haemorrhage occurs late in process of inflammation due to damage of endothelial lining of sinusoids leading to extravasation of blood into parenchyma. The livers of group I showed normal parenchyma but in group II, 46% of rats only show hemorrhage as arsenic was given to them for 30 days. In group III, hepatic parenchyma was normal with no hemorrhage.²⁹ Daqian Yang in his study gave As₂O₃ (arsenic trioxide) intraperitoneally in a dose of

3mg per kg for 2 weeks to rats and he found hemorrhage in liver histological examination. When olive oil was given to group III along with arsenic, due to its antioxidant effect it prevents the liver of rat to develop hemorrhage. In support of ameliorating effect of olive oil to improve hemorrhage of liver of our study, olive leave extract was used to improve hemorrhage in liver caused by carbendazim in a study done by Azab of Libya.²⁵

Conclusion

Olive oil protects histological changes caused by arsenic in liver of albino rats, which include sinusoidal dilation, congestion, pyknosis, necrosis and haemorrhage.

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