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Research Article

THE AMELIORATING EFFECT OF OLIVE OIL ON ARSENIC INDUCED HEPATIC HISTOMORPHOLOGICAL CHANGES OF ALBINO RATS

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ABSTRACT

Objective: To study the ameliorating effect of olive oil on histomorphological changes induced by arsenic in liver of albino rats.

Design of study: Randomized control trial

Place and duration: The research was carried out from 1st November 2017 to 30th November 2017 at NIH National institute of Health.

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, received olive oil of 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 has ameliorated the gross and microscopic qualitative changes induced by Arsenic in right lateral and left lateral hepatic lobes of Albino rats of group III as compared to group II rats, which received only arsenic. The gross parameters of colour, appearance, consistency and weight of livers were observed. The white colour of group II liver was improved to pale brown in group III due to use of olive oil. The use of olive oil in group III has prevented the complete loss of spongy normal appearance as they had few white patches along with normal spongy architecture. The olive oil had significantly prevented livers to become soft due to use of arsenic. The increase in hepatic weight due to arsenic was prevented by use of olive oil and was very close to the normal weight of control rats. Among qualitative microscopic parameters, congestion and necrosis was markedly reduced by use of olive oil in group III rats whereas haemorrhage was absent in group III.

Conclusion: The present study clearly demonstrates that olive oil ameliorates gross and qualitative histological changes caused by arsenic in liver which include colour, appearance consistency, weight, congestion, necrosis and haemorrhage.

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INTRODUCTION

The 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 produces toxic effects on liver. Arsenic, a "protoplasmic poison" interferes with mitosis, cell respiration, enzymes due to its effect on sulphhydryl group of cells^[2]. In Pakistan, arsenic contaminated groundwater is extensively utilized both for irrigation and drinking nearly in all areas of

Pakistan. Ground water of 32 districts of Punjab has been found to be contaminated with toxic level of arsenic.^[3] About 10 µg per liter arsenic is permissible in drinking water according to WHO guidelines, but the government of Pakistan says that 50 µg per liter is acceptable. This new study shows that 50-60 million people living in the Indus valley are drinking water which very likely exceeds their government's safe level.^[4] 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.^[7] Various antioxidants are available which can reduce the effect of arsenic on liver and

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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.^[8] Arsenic affects most of the organs involved in absorption, accumulation and excretion.^[9] Long-term exposure to inorganic arsenic can cause dysfunction of endocrine system^[10,11] nervous system^[12] and reproductive system^[13] and may also cause loss of body weight.^[6] Exposure to arsenic also causes, liver fibrosis, metabolic disorder such as diabetes,^[14,15] chronic lung disease, gangrene of toes,^[11] cancer of internal sites and skin.^[16] It has been found to have toxic effects on gonadal tissues of laboratory animals as well.^[13]

Various antioxidants have been used to ameliorate toxic effects of arsenic on various organs. Vitamin E, Ca and olive oil is used to improve Arsenic induced histological changes in ovary.^{[17],[8]} Antioxidants like L ascorbic acid, biochanin A, mentha piperita and aloe vera has been used to improve toxic effects of arsenic on liver of rats.^{[18]-[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.

MATERIAL AND METHODS

The experiment was carried on the basis of randomized control trial under supervision of animal house at NIH Islamabad from 1st November 2017 to 30th November 2017. Male albino 45 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 ERC 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
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

Appearance of liver

All livers of group I showed normal spongy appearance on cross section. In group II, 100 % of liver showed complete white appearance might be due to necrosis and in group III 100 % of liver showed few patchy white areas with normal spongy architecture. The simultaneous use of olive oil and arsenic has prevented the livers to become completely white in appearance. (Figure 1) The p value was 0.000 which was significant.



Figure 1 Gross appearance of livers of control and experimental groups

Colour of liver

The colour of all livers of rats of control group was dark brown as chocolate. In group II, 100% of the livers had white colour as cheese whereas 100 % of rats of group III had pale brown colour. The white colour might be due to necrosis caused by arsenic and pale brown colour showed olive oil has prevented the change in colour of rat livers caused by arsenic in group III. The p value was 0.000 which was significant.

Consistency of liver

The consistency of livers of control group was normal which was neither soft nor firm. 100% of livers of group II had soft consistency whereas 100% of livers of group III showed soft as well as normal consistency. The white part showed soft consistency whereas part of liver with normal spongy architecture had normal consistency. In this way, olive oil had significantly prevented the soft consistency of liver caused by arsenic in group III. The p value was 0.000 which was significant.

Weight of liver

Mean weight of rat liver in control group I was 7.68 g while in experimental group II it was increased to 10.19 g. In group III, mean weight was 7.83 g which is very close to the normal weight of livers of control group. This proves that olive oil has significantly reverted the increase in weight caused by arsenic in group III. The difference in mean weight of two experimental groups was significant but mean difference between control group I and III was insignificant which shows that weight of group III was reduced by use of olive oil such that it was almost same as normal weight of control group I.

Table 1. Distribution of mean weight of livers (grams) and multiple comparison of weights among control and experimental groups of Albino rats

Groups	Mean weight in gm	SEM	p value	Comparison	Mean difference	P value
Group I control	7.68	0.4459		I vs II	2.513	0.000
Group II experimental arsenic	10.19	0.4459		I vs III	0.149	0.940
Group III experimental arsenic and olive oil	7.83	0.4459	0.000	II vs III	2.363	0.000
<i>p value</i>			0.000*			

Groupwise distribution of congestion in sinusoids and central vein

The control group showed normal 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 4) p value was 0.000.

Table 2 Distribution of congestion in sinusoids and central vein among control and experimental groups

Groups	present N (%)	absent N (%)	Total	P value 0.000
Group I control	0(0 %)	15(100%)	15	
Group II experimental arsenic	15(100%)	0(0%)	15	
Group III experimental arsenic and olive oil	7(46.7%)	8(53.3%)	15	
Total	22	23	45	

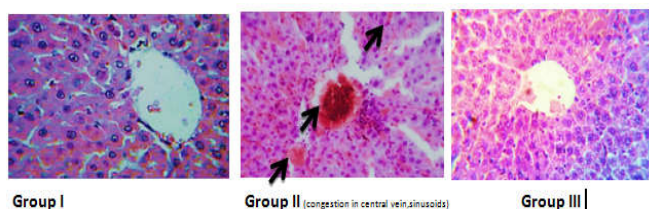


Figure 2 Group wise distribution of congestion of sinusoids and central vein Showing normal sinusoids and central vein in group I L7D,congestion in central vein and sinusoid in group II L4B,whereas no congestion in group III L3C.(H and E,X 40).(indicated by arrow heads in figure 2

Groupwise Distribution of Necrosis in Hepatic Parenchyma

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 .p value (0.00)

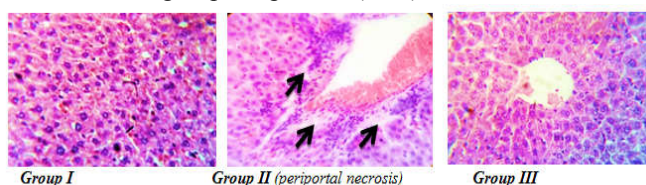


Figure 3 Group wise distribution of necrosis of hepatic parenchyma among rat groupsshow normal hepatic parenchyma in group IL7A2,presence of coagulative necrosis in periportal areas in group II L5B,and absence of necrosis in in group III L3A(H and E,X 40).(indicated by arrow head in group II figure 3

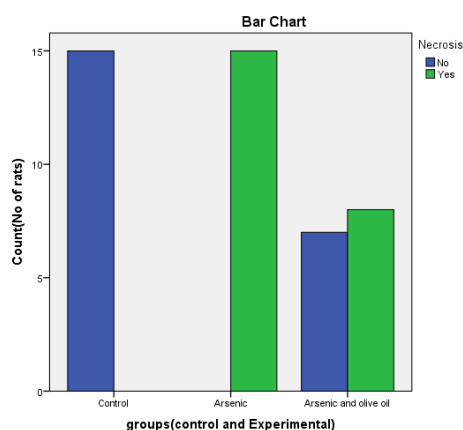


Figure 4 Bar chart showing distribution of necrosis among control and experimental groups

Group wise distribution of haemorrhage in hepatic parenchyma

In group I,hepatic parenchyma appeared to be normal,46% of rats in group II showed haemorrhage.In group III, olive oil has significantly improved haemorrhage as it was absent in all rats.(Table 4.5) (figure 5).The p value was 0.00 which is significant

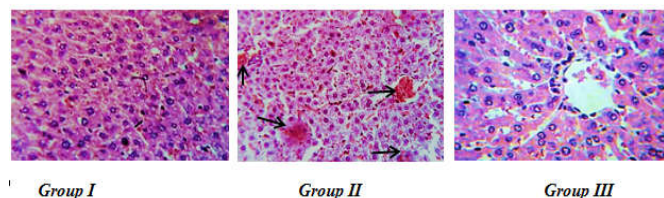


Figure 5 Group wise distribution of haemorrhage in hepatic parenchyma among groups

Table 3 Group Wise distribution of haemorrhage in hepatic parenchyma among control and experimental groups of albino rats

Groups	present N(%)	absent N(%)	Total	P value
Group I (control)	0(0%)	15(100%)	15	0.000*
Group II(experimental arsenic)	7(46.7%)	8(53.3%)	15	
Group III(experimental arsenic and olive oil)	0(0%)	15(100%)	15	
Total	7(15.6%)	38(84.4%)	45	

DISCUSSION

The liver, one of the vital organ perform various functions including detoxification of endo- and xenobiotic compounds, regulation of the plasma concentration of various metabolites, synthesis of most plasma proteins, synthesis of bile and production of hormone.^[21] Arsenic is one of the heavy metal which can cause liver diseases ,olive oil is one of antioxidant used in present study as an antioxidant for ameliorating arsenic induced histological changes.^[22]

In the present study, weight, appearance, colour and consistency were observed. Mean weight of rat liver in control group I was 7.68 g while in experimental group II it was increased to 10.19 g. In group III, mean weight was 7.83 g which is almost same as the normal weight of livers of control group. This proves that olive oil has significantly prevented the increase in weight caused by arsenic in group III. The increase in weight of liver is caused by arsenic due to the acute inflammation causing enlargement of hepatocytes and increase in diameter of hepatic sinusoids.^[23] The same result is supported by one of the studies conducted by Shafik in which the weight of rat livers was increased due to exposure to arsenic for 3 weeks.^[24] Liver weight shows a significant increase in another study by Adil due to hepatic toxicity induced by arsenic and improved weight gain by improving effect of naringin.^[25] A study conducted in Nigeria by Olusegun also showed an increase in liver and brain weight due to the combined exposure of arsenic and di- (ethylhexyl) phthalate.^[26] Against the result of this study, it was found that the weight of the liver did not change after 8 weeks of exposure to sodium arsenite in a study conducted by Souza *et al.*^[27] This is because a longer duration of exposure to arsenic causes chronic inflammation and fibrosis that cause the contraction of

the liver and decreases the weight. The use of olive oil has significantly maintained the normal weight of liver as it prevents the process of inflammation of liver caused by arsenic due to its antioxidant effect.

The appearance and colour of liver was observed in control and experimental groups of study. All livers of group I showed normal spongy appearance on cross section. In group II, all livers showed complete white necrosed appearance and in group III 100 % of liver showed few patchy necrosed areas with normal spongy architecture. The white necrosed appearance is due to necrosis which is the result of inflammation caused by toxins or viruses.^[28] The simultaneous use of olive oil and arsenic has improved the complete white necrosed appearance to patchy necrosed appearance in group III.

The colour of all livers of rats of control group was dark brown. In group II, 100% of the livers had white colour whereas 100 % of rats of group III had pale brown colour. The white colour is due to necrosis caused by arsenic. In one of the studies conducted by Gyorgy Paul, the inflammatory appearance of the liver showed variation in the appearance of the liver, from speckled mottled necrosis to necrotic white appearance. The variations in the color of patches from light yellow, dark red and brown were remarkable.^[29] The pale brown colour of livers showed that olive oil has significantly reversed the change in colour of rat livers caused by arsenic in group III.

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.^[30] 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.^[31] 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 leave 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.^[32]

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.^[34] 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.^[35] The use of olive oil in group III has improved necrosis in our study. Metin Ogun, used oleuropin 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 oleuropin group showed necrosis in only 1 mouse.

The haemorrhage occur 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 haemorrhage as arsenic was given to them for 30 days. In group III, hepatic parenchyma was normal with no haemorrhage.^[36] 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 haemorrhage 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 haemorrhage. In support of ameliorating effect of olive oil to improve haemorrhage of liver of our study, olive leave extract was used to improve haemorrhage in liver caused by carbendazim in a study done by Azab of Libya.^[32]

CONCLUSION

The present study clearly demonstrates that olive oil ameliorates histomorphological changes caused by arsenic in liver which include colour, appearance, consistency, weight, congestion, necrosis and haemorrhage.

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