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# LAGENARIA SICERARIA;

## LAGENARIA SICERARIA AS AMELIORATIVE AGENT AGAINST INFLAMMATION AND FIBROSIS INDUCED BY SODIUM ARSENITE ON THE HISTOMORPHOLOGY OF RAT LIVER.

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**ABSTRACT… Objectives:** To observe the effect of lagenaria siceraria on inflammation and fibrosis brought about by arsenic in liver of Sprague Dawley rat. **Study Design:** Laboratory based randomized control trial. **Place and Duration of Study:** This experiment was performed at Department of Anatomy, Army Medical College Rawalpindi in co-operation with National Institute of Health (NIH) Islamabad for eight weeks (1st March 2017 to 25th April 2017). **Material and Methods:** Fifty Sprague Dawley rats (both male and females housed separately) were carefully chosen and distributed randomly into five groups, each consisting of 10 animals. A and B were the control groups whereas C, D and E served as experimental groups. During the first four weeks, experiment groups C, D and E were given a dosage of 5milligram/kilogram body weight of sodium arsenite. At the end of four weeks, animals from control group A and experimental group C were dissected and liver samples were processed for microscopic studies. In the next 4 weeks, group D animals were set aside without any further intervention. At that time, sodium arsenite at a dose of 5 milligram/kilogram body weight and lagenaria siceraria at a dose of 100 milligram/kilogram bodyweight were administered to group E animals. Group B animals served as control for experimental groups D and E. At the end of these 4 weeks animals of groups B, D and E were dissected. **Results:** Experimental group C developed moderate grade fibrosis and inflammation (grade 2 to 3) as compared to group A. Degree of inflammation and fibrosis was mild to moderate (grade 1 to 2) in group D. There was no inflammation and fibrosis (Grade 0 to 1) in group E. Group B served as a control for group D and E. **Conclusion:** Inflammation and fibrosis developed in the liver of adult rats when they were subjected to sodium arsenite even for a brief calculated period. Simultaneous administration of lagenaria siceraria can shield and diminish the toxic effects of arsenic. Oxidative potential and immunomodulatory properties and presence of flavonoid like substances renders lagenaria siceraria to act as ameliorative against this fibrosis and inflammation in liver lobules and surrounding area of portal triads.

**Key words:** Fibrosis, Inflammation, Lagenaria Siceraria, Sodium Arsenite.

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## INTRODUCTION

Toxicity and heavy metals go hand in hand. Arsenic, a heavy metal is notorious for its carcinogenic abilities and if consumed in higher doses is actually lethal.[1] As this arsenic enhances growth and body mass, it is mixed on purpose with chicken feed. A large number of chicken are raised on arsenic so as to rouse their growth on least food intake, it at the same time adds a healthy color to meat.[2] Food and drug administration (FDA) gave their reports saying that tests performed on chicken revealed that half of the tested chicken had arsenic in their liver.[3] On the other hand, Roxarsone a preparation which comprises of lethal arsenic gives fresh pink color to the meat that is why it is supplemented in chicken feed.[4] Arsenic absorption is best via the air passages and alimentary canal. After absorption of inorganic arsenic in the liver, it maximally goes through methylation, to transform into monomethylarsonic acid and dimethylarsinic acid. Whereas the residual inorganic arsenic is mainly removed through urine. If the soluble inorganic arsenic is absorbed in quantities less than 1000$\mu$g, majority of the ingested dose is expelled in the urine during the next 2–3 days.

Elimination half-life is delayed if the ingested dose is higher.[5] Liver and kidney are primarily effected by the arsenic induced toxins however, the liver is at a higher risk than kidneys because of high levels of arsenic in it. Apoptosis within the liver by up regulation of pro-apoptotic proteins is produced by arsenic-induced oxidative stress.[6]

Quite a number of medical help providers use herbal medications for the healing and cure of various ailments.[7] In the innovative works of Ayurvedic and Unani medicine there exists plenty of data and information about the benefits of herbal prescriptions.[8] Survey conducted by world health organization (WHO) deduced that majority of population of developing countries exclusively have faith in traditional medication for their main health care prerequisites.[9] Not only this but some of the very essential lifesaving preparations have been derived from herbs which are in use in modern day medical techniques. Herbal medicines are considered all across the globe to be less injurious and nontoxic to humans as compared to synthetic preparations.[10] Keeping this in mind the research organizations throughout the world are involved in isolating plants having medicinal capabilities. The customary pharmaceutical system quotes these herbal therapies for the management of variety of ailments. Importance of food in the treatment of diseases has been thoroughly stressed in Ayurveda.[11]

Practitioners of this era have also recognized the impact of dietary articles as nutraceutical fundamentals, in the management of long-lasting ailments.[12] A herb called Lagenaria siceraria (bottle gourd) is a commonly grown vegetable which is used in customary medication for the management of illnesses and complaints. This herb is a unique fruitlet possessing combination of all the necessary elements needed for steady and virtuous well-being of humans. Lagenaria siceraria as a matter of course is utilized for its cardio protective, cardio tonic and general tonic properties and is also used as substitute to purgative and diuretic.[7,12] Ethanolic extract of Lagenaria siceraria was tested against $CCl_4$ hepatotoxicity where they showed ameliorative effects.[7,8] Lagenaria siceraria is also a pharmacological plants which is thought to possess immunomodulatory capability of enhancing the immune system of the host, this helps in confronting the antigens.[13] Latest surveys have led the researchers to comprehend the importance of this herb in human well-being because of the fact that it can provide a variety of roles including anthelmintic, antibacterial, antifungal, immunomodulatory, antiallergic, analgesic, anti-inflammatory, antioxidant and hepatoprotective capabilities.[14,15,16]

## MATERIAL AND METHODS

This study was carried out at Army Medical College, Rawalpindi in alliance with National institute of Health (NIH), Islamabad by the consent of Ethical Committee of the Army Medical College Rawalpindi. Experiment was conducted at the Department of Anatomy, Army Medical College, Rawalpindi. Chemical was bought from Sigma Aldrich (Product no.133027-500G) whereas the Lagenaria siceraria was bought from local bazaar for this experiment and was made into fine powder form by air drying then grinding. Adult male and female Sprague Dawley rats fifty in number weighing 250 – 380 gm, 9-11 weeks of age were taken for this study and both sexes were kept in separate cages. The well-ventilated rooms in which the rats were kept, maintained a temperature range of 20 - 26°C, with a 12 hour dark-light sleep cycle throughout the duration of experiment.[17] Standard laboratory diet was provided by NIH in which water was given at ad libitum. Calculated amount of chemical and dried powder form of lagenaria siceraria mixed with normal saline, were administered via oral gavage needle.

Group A and B animals were controls, on the other hand, C, D and E were experimental groups. Study was divided in two phases, in the first phase of 4 weeks the experimental groups were given sodium arsenite 5mg/kg/day.[18] At the end of the first phase, animals from control group A and experimental group C were sacrificed and specimens were prepared for microscopic evaluation to study the immediate effects of arsenic toxicity. In the second phase, group D was retained without any intervention just to see

whether the toxic effects were self-limiting. At the same time, group E was given sodium arsenite 5mg/kg/day and lagenaria siceraria 100mg/kg/day simultaneously.[19] At the end of the second phase, the animals of group B, D and E were sacrificed, specimens were taken out, prepared and processed. Slides of these specimens were prepared and stained with haematoxylin and eosin (H&E), and by Masson trichrome to assess the fibrosis under light microscope. For evaluation of level of inflammation H & E stained slides of the specimens were prepared and studied. Each sample was assessed for grade of fibrosis and inflammation by using Knodell and Ishaak scoring[20-22] in 3 successive HPF for both H&E and Masson trichrome stained specimen. Statistical package for social sciences version 22 was used to evaluate the data, the parameters were expressed as percentages and analysis of groups were done by using cross tabs followed by chi-square test. P-value of ≤0.05 was considered significant, besides that mean ± standard deviation is presented in Table-I and II.

## RESULTS

Fifty Sprague Dawley rats with an average weight of 284 gm, 9-11 weeks of age, were used in this study. On microscopic examination of liver, control groups A and B displayed typical hepatic architecture with cords of hepatocytes in form of scattering rays from the central vein and the rays of radiating cords are limited by thin connective tissue layer establishing hepatic lobule. Specimens of group C exhibited moderate grade inflammation categorized by the damage to architecture of **liver** cells and the presence of inflammatory cells in the hepatic parenchyma, at the same time moderate degree of fibrosis was also visible. Group D specimens showed mild inflammation and fibrosis. Contrary to this, none of group E specimens showed inflammation or fibrosis. The inter group comparison for fibrosis revealed that 100 % of the experimental animals did not reveal any fibrosis, mild degree of fibrosis was seen in 100 % of animals in group C and 50% of animals in group D, while moderate and marked fibrosis is not reported in any of the groups. A p-value of < 0.001 which is considered statistically highly significant Table-I. At the same time the intergroup comparison for inflammation revealed that none of the animals in group A and B, 100% of the animals in group C showed mild inflammation whereas 50 % of the animals in group D revealed mild inflammation, whereas moderate to severe inflammation was not observed in any of the animals from all the experimental groups. A p-value<0.001 which again is statistically highly significant Table-II.

**a) Normal looking periportal area(H & E stain)**
**b) Periportal inflammation (H & E stain)**
**c) Periportal area with no fibrous change (Masson's trichrome stain)**
**d) Periportal area showing fibrosis (Masson's trichrome stain)**

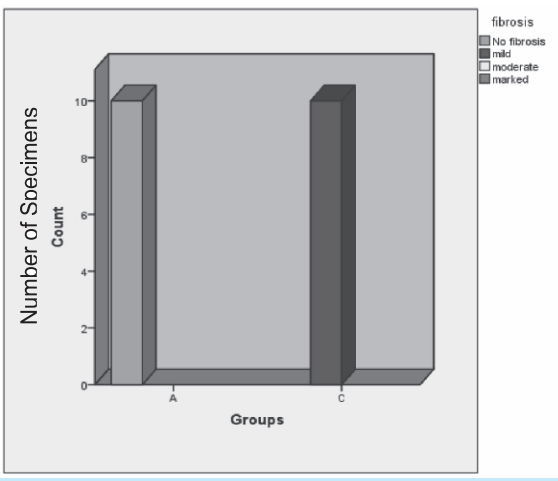


**Figure-1. Bar chart showing intergroup comparison of Fibrosis of liver among control group A and experimental group C**

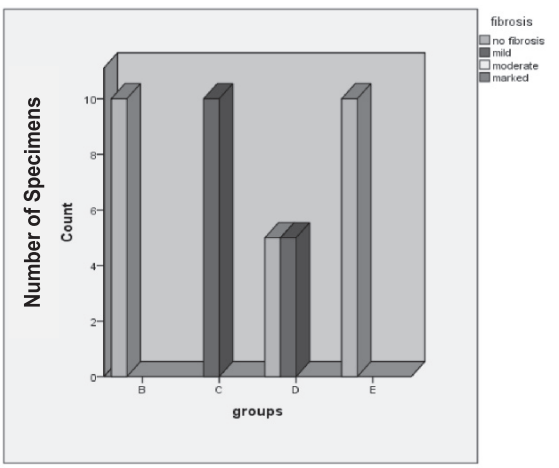


**Figure-2. Cluster bar chart showing intergroup comparison of fibrosis of liver among control group B and experimental groups C, D and E**

| Parameters | Findings | Group A | Group B | Group C | Group D | Group E | P- value |
|---|---|---|---|---|---|---|---|
| Fibrosis | Absent | 10 (100%) | 10 (100%) | 0 (0%) | 5 (50%) | 10(100%) | < 0.001* |
| | Mild | 0 (0%) | 0 (0%) | 10 (100%) | 5 (50%) | 0 (0%) | |
| | Moderate | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | |
| | Marked | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | |

**Table-I. Frequency and percentages of fibrosis in the liver in control groups A, B and experimental groups C, D and E**
p-value ≤ 0.05 is statistically significant * = highly significant

| Parameters | Findings | Group A | Group B | Group C | Group D | Group E | P-Value |
|---|---|---|---|---|---|---|---|
| Inflammation | Absent | 10 (100%) | 10 (100%) | 0 (0%) | 5 (50%) | 10 (100%) | < 0.001* |
| | Mild | 0 (0%) | 0 (0%) | 10 (100%) | 5 (50%) | 0 (0%) | |
| | Moderate | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | |
| | Severe | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | |

**Table-II. Frequency and percentages of inflammation of control group A, B and experimental groups C, D and E**
p-value ≤ 0.05 is statistically significant * = highly significant

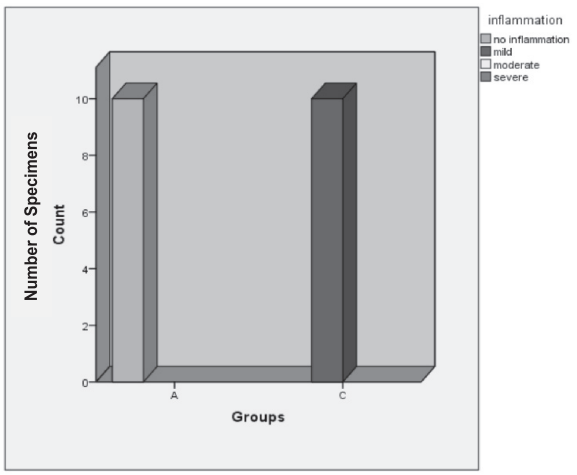


**Figure-3. Bar chart showing intergroup comparison of inflammation among control group A and experimental group C**

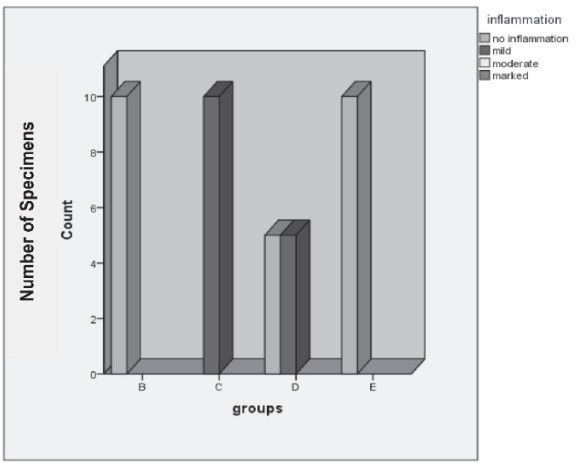


**Figure-4. Cluster bar chart showing intergroup comparison of inflammation among contol group B and experimental groups C, D and E**

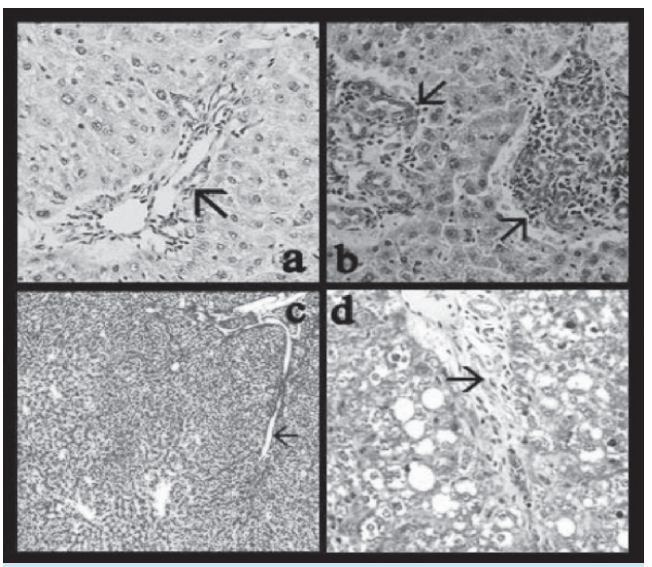


**Figure-5. Figure showing**

## DISCUSSION

Sodium arsenite coming in contact through various routes has hostile effects on numerous tissues and organs, leading to ill health.[5] Consequently, this experiment was planned to examine the influence of sodium arsenite on the liver of adult rats and whether concomitant intrusion of lagenaria siceraria can amend the influence. In the present study, introduction of mild doses of sodium arsenite for a calculated period of time modified the hepatic architecture inducing inflammation and fibrosis in the lobules and in the vicinity of portal triads. Control groups A and B showed normal hepatic parenchyma with no signs of fibrosis and inflammation. Group C revealed moderate grade fibrosis and inflammation. Group D showed mild to moderate

degrees of inflammation and fibrosis while group E showed an image similar to that of the control groups A & B. The changes in the specimens were highly significant when inter group comparison were done between the groups A, B, C, D and E. These effects were also plotted on bar graphs presenting contrast of fibrosis in group A and C (Figure-1 & 3), and amongst B, C, D and E (Figure-2 & 4). The results were in alliance with the works done in the past.[23]

Toxic effects of arsenic on kidney and liver are mainly oxidative stress, apoptosis, and up regulation of transcription factors such as AP-1, ATF-2, and Elk-1.[24] Studies in the past depicted that when the animals under study were introduced to arsenic they responded by developing fibrosis and at the same time oxidative stress was induced in the hepatocytes resulting in inflammation.[25] Heavy metals have hepatotoxic potential due to production of oxidative stress to the tissue, lagenaria siceraria has remarkable oxidative capability and is in a position to reverse the oxidative stress and concomitantly can minimize the inflammation in the interstitium and area of portal triads.[26,27]

Lagenaria siceraria on biochemical evaluation was found to have flavonoids. Plants which have flavonoids have an impact on arachidonic acid metabolism and are thought to have anti-inflammatory, cardio protective and anti-allergic effects[28,29] Present study also revealed that when lagenaria siceraria was simultaneously given to the animals who were exposed to heavy metal toxicity the process of inflammation and fibrosis was reverted (Table-I&II). Results were also plotted in bar charts to study the intergroup comparison. Liver usually gets involved in toxic phenomenon because of the fact that most of the materials including the toxic elements are ultimately taken up by liver for metabolism, which renders the organ to be more prone to toxic effects.[30] Main enzymes of liver are aspartate, aminotransferase and alanine aminotransferase, as a result of injury to cell due to arsenic exposure, increased levels of these enzymes are then liberated into the blood stream which confers to hepatotoxicity.[31] Many epidemiological studies outline a direct relationship between continued arsenic contact through drinking water and deranged liver functions, hepatoportal sclerosis, enlarged liver, fibrosis ending up in cirrhosis, ascites.[32]

## CONCLUSION

This experiment proposes that Sodium arsenite is able to disrupt the architecture of liver associated with it is inflammation and fibrosis in the lobules as well as in the vicinity of portal triad and concomitant administration of lagenaria siceraria will have ameliorative effect on the liver architecture.

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## CONFLICT OF INTEREST

No conflict of interest amid their impression was reported by any of the authors.



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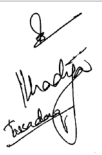
| 1 | Saima Qureshi | 1st AUthor |  |
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