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Antidote effect of Honey against arsenic induced toxicity in Charles Foster rats

Manisha Kiran[1], Shobha Shrivastava[2], Arun Kumar[3]*

[1] Department of Zoology, Patna University, Patna, Bihar, India.

[2] Department of Zoology, Patna Women's College, Patna University, Patna, Bihar, India.

[3] Mahavir Cancer Sansthan and Research Centre, Patna, Bihar-801505, India.

*Corresponding Author: E-mail: drarunk31@gmail.com, Cellphone: +91-9334740800, Orcid I.D.: https://orcid.org/0000-0002-8946-5909

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Summary

Introduction: Arsenic poisoning in the groundwater in recent times has become a major problem in the entire world. The exposed subjects exhibit typical symptoms of arsenicosis such as skin manifestations, gastrointestinal disorders, neurological disorders, hormonal disorders etc. **Objective:** The present study is focused to combat the deleterious effect of arsenic toxicity in animal models using honey. **Materials and Methods:** The animals (Charles Foster rats) were treated with Sodium arsenite at the dose of 8 mg per Kg body weight for 90 days to make arsenic model and upon these arsenic pre-treated rats honey at the dose of 200 mg per Kg body weight (1:1) was administered for 60 days to study the antidote effects. After the entire treatment, rats were sacrificed and their blood samples were obtained and analysed for haematological and biochemical and histopathological study. **Results:** The study shows that arsenic induced toxicity caused severe damage to the rats at the haematological level such as decrease in the RBC counts, WBC counts, haemoglobin percentage, and biochemical level such as increase in the levels of SGPT, SGOT, ALP, bilirubin, urea, uric acid, creatinine, and lipid peroxidation. There was also high magnitude of degeneration observed at the histopathological level in the liver and kidney tissues. But, there was significant normalization in the honey on arsenic pretreated group of rats at all the respective studied parameters. **Conclusion:** The studied parameters denote that honey possesses antidote properties against arsenic induced toxicity and can be used for human purpose after dose titration as antidote.

Keywords: Sodium arsenite, honey, antidote effect, Charles Foster Rats.

Resumen

Efecto antídoto de la miel contra la toxicidad inducida por arsénico en ratas Charles Foster

Introducción: El envenenamiento por arsénico en las aguas subterráneas en los últimos tiempos se ha convertido en un problema importante en todo el mundo. Los sujetos expuestos presentan síntomas típicos de arsenicosis, como manifestaciones cutáneas, trastornos gastrointestinales, trastornos neurológicos, trastornos hormonales, etc. **Objetivo:** El presente estudio se centra en combatir el efecto nocivo de la toxicidad del arsénico en modelos animales que utilizan extracto de plantas medicinales. **Materiales y métodos:** Los animales (ratas Charles Foster) fueron tratados con arsenito de sodio a una dosis de 8 mg por Kg de peso corporal durante 90 días para hacer un modelo de arsénico y, sobre estas ratas pretratadas con arsénico, miel a una dosis de 200 mg por Kg. Se administró peso corporal (1:1) durante 60 días para estudiar los efectos del antídoto. Después de todo el tratamiento, las ratas fueron sacrificadas y se obtuvieron y analizaron muestras de sangre para su estudio hematológico, bioquímico e histopatológico. **Resultados:** El estudio muestra que la toxicidad inducida por arsénico causó daños graves a las ratas a nivel hematológico, como disminución en los recuentos de glóbulos rojos, recuentos de glóbulos blancos, porcentaje de hemoglobina y nivel bioquímico, como aumento en los niveles de SGPT, SGOT, ALP, bilirrubina. , urea, ácido úrico, creatinina y peroxidación lipídica. También se observó una alta magnitud de degeneración a nivel histopatológico en los tejidos del hígado y el riñón. Pero hubo una normalización significativa en el grupo de ratas pretratadas con miel y arsénico en todos los parámetros estudiados respectivos. **Conclusión:** Los parámetros estudiados denotan que la miel posee propiedades antídoto contra la toxicidad inducida por arsénico y puede ser utilizada para fines humanos después de la titulación de dosis como antídoto.

Palabras clave: arsenito de sodio, miel, efecto antídoto, ratas Charles Foster.

Resumo

Efeito antídoto do mel contra a toxicidade induzida por arsênico em ratos Charles Foster

Introdução: O envenenamento por arsênico nas águas subterrâneas tornou-se nos últimos tempos um grande problema em todo o mundo. Os sujeitos expostos apre-

sentam sintomas típicos de arsenicose, como manifestações cutâneas, distúrbios gastrointestinais, distúrbios neurológicos, distúrbios hormonais, etc. **Objetivo:** O presente estudo tem como objetivo combater o efeito deletério da toxicidade do arsênico em modelos animais utilizando extrato de planta medicinal. **Materiais e Métodos:** Os animais (ratos Charles Foster) foram tratados com arsenito de sódio na dose de 8 mg por Kg de peso corporal durante 90 dias para fazer modelo de arsênico e sobre estes ratos pré-tratados com arsênico mel na dose de 200 mg por Kg peso corporal (1:1) foi administrado por 60 dias para estudar os efeitos do antídoto. Após todo o tratamento, os ratos foram sacrificados e suas amostras de sangue foram obtidas e analisadas para estudo hematológico, bioquímico e histopatológico. **Resultados:** O estudo mostra que a toxicidade induzida pelo arsênico causou danos graves aos ratos no nível hematológico, como diminuição na contagem de glóbulos vermelhos, contagem de leucócitos, porcentagem de hemoglobina e nível bioquímico, como aumento nos níveis de SGPT, SGOT, ALP, bilirrubina, uréia, ácido úrico, creatinina e peroxidação lipídica. Houve também alta magnitude de degeneração observada em nível histopatológico nos tecidos do fígado e dos rins. Mas houve uma normalização significativa no mel do grupo de ratos pré-tratados com arsênico em todos os respectivos parâmetros estudados. **Conclusão:** Os parâmetros estudados indicam que o mel possui propriedades antídoto contra a toxicidade induzida pelo arsênico e pode ser utilizado para fins humanos após titulação da dose como antídoto.

Palavras-chave: Arsenito de sódio, mel, efeito antídoto, ratos Charles Foster.

Introduction

Groundwater arsenic poisoning in the recent times has caused serious health hazards worldwide. A wide population of about 300 million are exposed to arsenic through groundwater poisoning. Moreover, in Asia, India and Bangladesh share major river channels Ganga-Meghna-Brahmaputra plains where an estimated 155 million are exposed to groundwater arsenic poisoning. India and Bangladesh have the cases of arsenic poisoning in the different habitations about 70 million and 85 million population respectively. This arsenic poisoning number is contributed as half of the world's arsenic exposed population. In India, the groundwater arsenic poisoning is being majorly reported from states – West Bengal, Assam, Bihar and Uttar Pradesh. Moreover, this is the chunk area where the population of about 510 million reside in the different habitations [1-6].

In Bihar, Bhojpur was the first district in 2002, when arsenic poisoning was reported for the first time and presently, 22 districts of state are affected with groundwater arsenic poisoning [7-9]. The exposed population exhibit typical symptoms of arsenicosis such as skin manifestations, neurological disorders, hormonal imbalance, reproductive disorders, gastrointestinal disorders, cardiovascular disorders, diabetes, and loss of appetite and disease of cancer [10-14]. Moreover, in this arsenic exposed area the magnitude of disease burden has increased many folds in the recent times. Among the cancer types – skin cancer, lung cancer, colorectal cancer etc. have been reported from the arsenic exposed population [15-23]. Hence, it becomes very important to discover natural products which can be recommended to the arsenic exposed population which can reduce the disease burden in them.

Plethora of medicinal plants have been documented which have therapeutic effect on arsenic induced toxicity in animal models [24-26], but their products have not reached to the exposed population till date. The use of honey has been extensively used against various types of poisons [27], anti-inflammation, anti-apoptosis, anti-fibrosis [28], cardiovascular diseases such as anti-arthrosclerosis effects [29], anti-neuroinflammatory effect [30], and other diseases [31, 32]. Hence, the present study is focused on the usage of natural wild supplement such as honey. Honey has been used since many years as source of energy, for wound healing, anti-bacterial, anti-viral and as natural skin rejuvenators [33-43].

Hence, the present study aims to discover the antidote effect of natural honey against the arsenic induced model on Charles Faster rats and to validate the efficacy of the honey against arsenic induced toxicity.

Materials And Methods

Animals

For the entire experimentation, n=24, male Charles Foster rats, 8 weeks old of weight about 150-180 g were provided from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India. The animals were acclimatized in laboratory for 2 weeks before the start of the experiment. All the laboratorial conditions were maintained with 12 hours of light and dark cycles and room temperatures were maintained at 22 ± 2 °C with adjusted humidity. The animals had free access to food and water *ad libitum*.

Chemicals

For the experiment, to induce the toxic models in rats, Sodium arsenite (98.5%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736V, PCode 1001683292 as the chemical were utilized. The permissible dose of sodium arsenite was used as 8 mg/Kg/body weight per day for 90 days to make the arsenic model as per the previous study [23].

Preparation of honey dose

Raw wild honey was obtained from the local market and its purity was tested in the laboratory (by vinegar method and light microscopy). The dose of the honey was calculated after LD_{50} estimation and was titrated to 200 mg/Kg body weight per day. The dose was prepared by 1:1 ratio mixed in water as its viscosity was very high.

Experimental Design

The experimental animals were majorly disintegrated into 04 groups and each group consisted of six rats and were divided in following groups - **Group I: Normal Control group**; **Group II: Arsenic treated group** – The rats were treated orally with sodium arsenite at a dose of 8 mg/Kg body weight/day for 90 days; **Group III: Honey administered group**- Rats were pretreated with sodium arsenite 8mg/Kg body weight/day for 90 days followed by administration of honey – 200 mg/Kg body weight/day for 60 days. After the completion of the entire experiment, animals from each group were properly anaesthetized with 50 mg/Kg ketamine hydrochloride (intra-peritoneally) and sacrificed. The dissected animals' blood samples were collected through the orbital puncture for serum separation and were stored properly for the biochemical assays such as -Liver function tests, kidney function tests and lipid peroxidation. Furthermore, their tissue samples such as liver and kidney were properly preserved in the 10% formalin fixative and were processed later.

Haematological study

For the haematological study – RBC counts and WBC counts the Neubauer's chamber was utilized, while for the estimation of haemoglobin, hemoglobinometer was utilized using the Sahli's method.

Biochemical analysis

For the biochemical study, standard kit was used (Coral crest) by the Spectrophotometer (UV-Vis) (UV-10, Thermo Fisher, USA) method. The biochemical parameters used in the present study were - Liver Function Tests- Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) estimated

through the method of [44], Alkaline Phosphate (ALP) assay by the method of [45], total bilirubin activity by method of [46]. The Kidney Function Tests (KFT) were assayed as urea by the method of [47, 48], creatinine assay by the method of [49], and uric acid assay by the method of [50].

Histopathological study

The dissected tissues – liver and kidney which were fixed in 10% formalin for 24 hours were then processed through the series of graded alcohol and were embedded into paraffin blocks. Utilizing the digital microtome (Themo-Fisher), fine sections of about 5 μm thickness were cut and were processed for double staining method with hematoxylin and eosin (H&E). For the microscopic observations, the stained slides were viewed under the light microscope [51].

Lipid Peroxidation (LPO)

For the lipid peroxidation method, thiobarbituric acid reactive substances [TBARS] are the best markers for the evaluation. The assay was evaluated through the double heating method [52], based on the principle of spectrophotometric measurement of colour reproduced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this study, 0.5 mL of serum were mixed with 2.5 mL of 100 g/L solution of Trichloroacetic acid (TCA) and then were properly centrifuged at 3000 rpm for 10 minutes, it was then heated in the water bath at 90 °C for 15 minutes. After cooling at the room temperature, the mixture was re-centrifuged at 3000 rpm for 10 minutes and the supernatant were obtained. The 2 ml of the supernatant were measured and were mixed with 1 mL of 6.7 g/L freshly prepared TBA solution in the test tube which was further heated in water bath at 90 °C for 15 minutes and left for cooling at the room temperature. The final absorbance was measured using UV-vis spectrophotometer (Thermo Scientific UV-10, USA) at 532 nm.

Statistical analysis

The results presented in the study were the Mean ± Standard Error (SE) for six rats' individual groups and total variation represented in a set of data which were analyzed through one-way Analysis of Variance (ANOVA). The differences among mean variance were analyzed by applying Dunnett's 't' test at 99.9% ($p < 0.05$) confidence level. The final calculations were performed utilizing the GraphPad Prism Program 5.0 (GraphPad Software, Inc., San Diego, USA).

Results

Haematological Assay

There were significant changes observed in the studied groups in the haematological parameters –

a. **RBC Counts:** In the arsenic treated group there was significant decrease ($p<0.005$) in the RBC counts in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the RBC counts after the administration of honey to the arsenic pre-treated rat group (Figure 1).

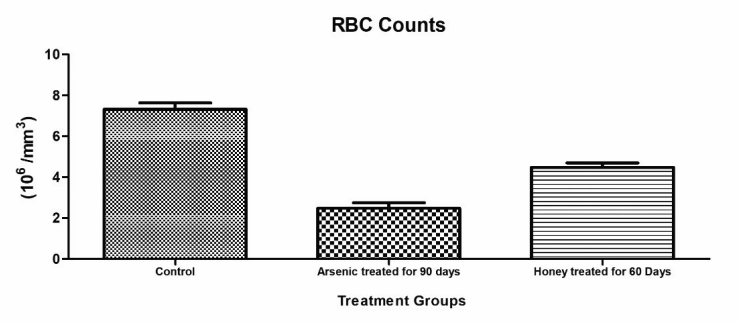

Figure 1. RBC counts of the treated groups (One way ANOVA Test in various group of rats (n=6), values are displayed as Mean ± SE)

b. **WBC Counts:** In the arsenic treated group there was significant decrease ($p<0.005$) in the WBC counts in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) increase in the WBC counts after the administration of honey to the arsenic pre-treated rat group (Figure 2).

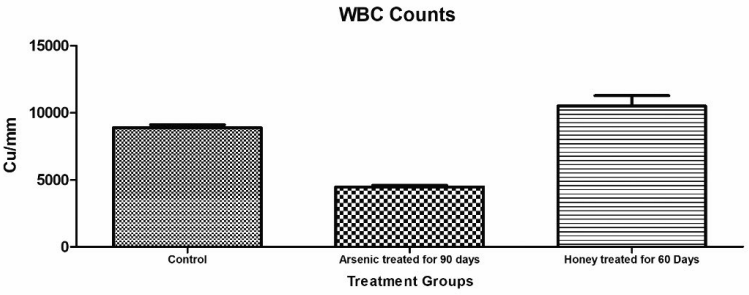

Figure 2. WBC counts of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

c. **Haemoglobin Percentage:** In the arsenic treated group there was significant decrease ($p<0.005$) in the haemoglobin percentage in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) decrease in the haemoglobin percentage after the administration of honey to the arsenic pre-treated rat group (Figure 3).

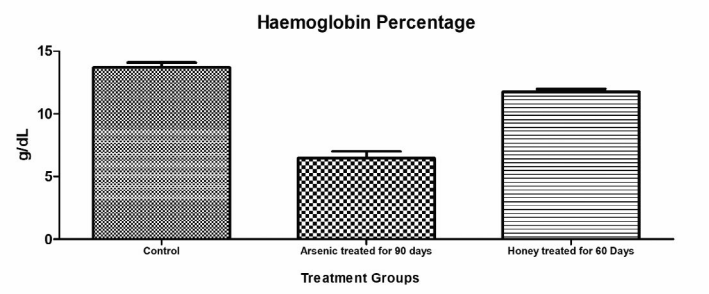

Figure 3. Haemoglobin percentage of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

Biochemical Study

There were significant changes observed in the biochemical parameters of the studied groups –

1. **SGPT Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the SGPT levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the SGPT levels after the administration of honey to the arsenic pre-treated rat group (Figure 4).

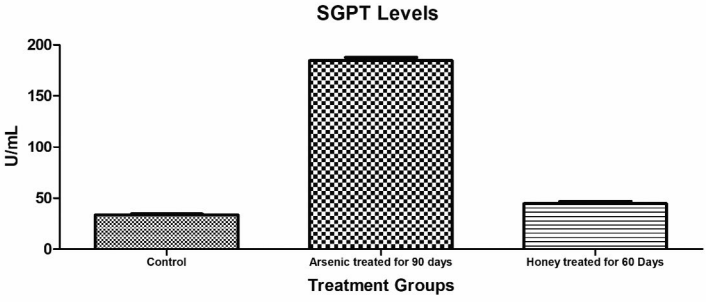

Figure 4. SGPT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

2. **SGOT Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the SGOT levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the SGOT levels after the administration of honey to the arsenic pre-treated rat group (Figure 5).

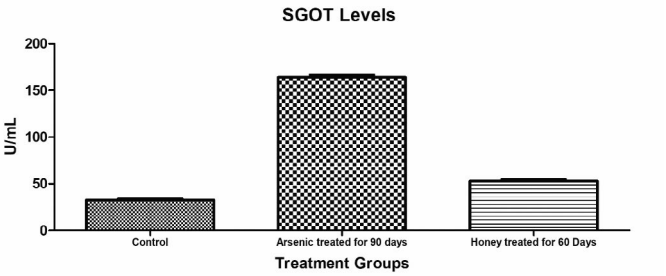

Figure 5. SGOT Levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

3. **Alkaline Phosphatase (ALP) Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the ALP levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the ALP levels after the administration of honey to the arsenic pre-treated rat group (Figure 6).

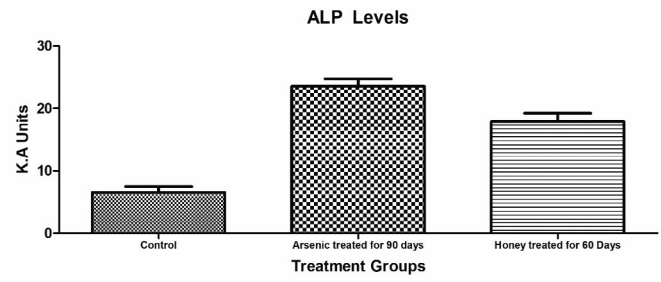

Figure 6. Alkaline phosphatase Levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

4. **Bilirubin Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the bilirubin levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the bilirubin levels after the administration of honey to the arsenic pre-treated rat group (Figure 7).

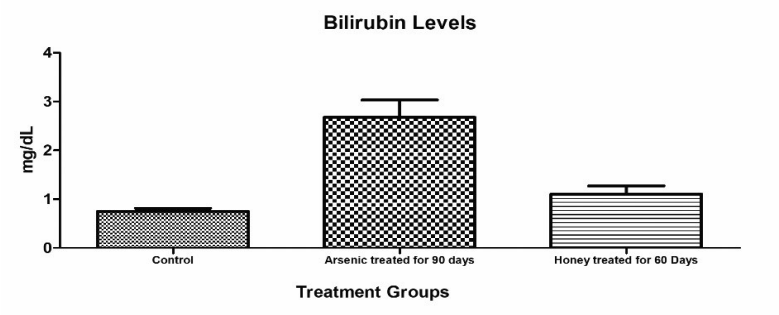

Figure 7. Bilirubin Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

5. **Urea Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the Urea levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the Urea levels after the administration of honey to the arsenic pre-treated rat group (Figure 8).

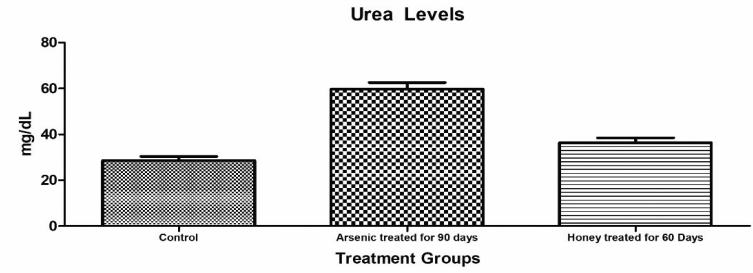

Figure 8. Urea Levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

6. **Uric Acid Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the Uric acid levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the uric acid levels after the administration of honey to the arsenic pre-treated rat group (Figure 9).

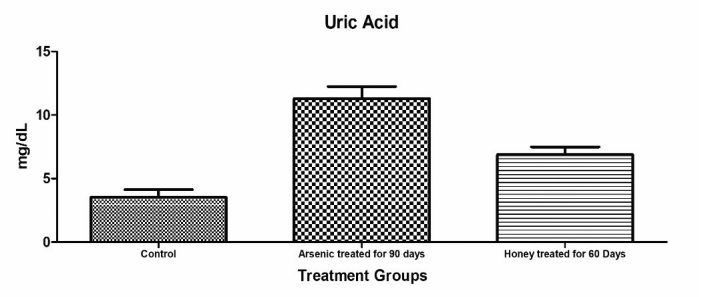

Figure 9. Uric acid Levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

7. **Creatinine Assay:** In the arsenic treated group there was significant increase ($p<0.005$) in the creatinine levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the creatinine levels after the administration of honey to the arsenic pre-treated rat group (Figure 10).

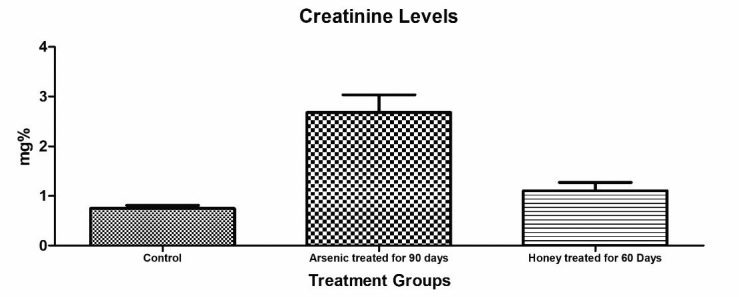

Figure 10. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

8. **Lipid Peroxidation (LPO) Assay:** In the arsenic treated group, there was significant increase ($p<0.005$) in the LPO levels in arsenic treated group of rats as compared to the control group. There was significant ($p<0.005$) normalization in the LPO levels after the administration of honey to the arsenic pre-treated rat group (Figure 11).

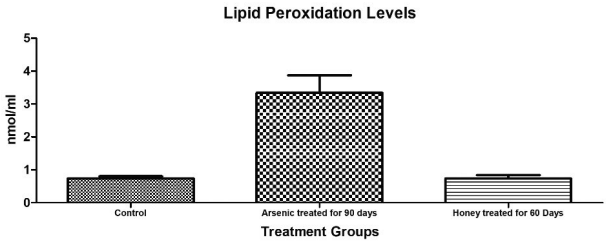

Figure 11. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

Histopathological Study

There were significant changes observed in the histopathological study in the studied groups – The liver histopathological sections showed normal architecture of hepatocytes with central vein. The hepatocytes were well arranged in the sinusoids denotes the normal functioning of the liver cells (Figure 12A). The arsenic treated rat liver section showed high grade of degeneration in the hepatocytes with pyknotic nuclei. There was manyfolds increase in the number of Kupffer cells denotes the macrophagic activity. Moreover, the endothelial cells of central vein membrane have ruptured severely by which haemorrhage in the sinusoidal spaces can be observed. Vacuolations in the sinusoidal spaces were also observed in the section. (Figure 12A). But after the administration with honey for 60 days, there has been significant restoration observation in the hepatocytes, the central vein and the sinusoids. The hepatocytes were well arranged in the sinusoids with the normal functioning. Moreover, no Kupffer cells were observed denotes the normal functioning of the liver. (Figure 12C & D).

The kidney histopathological sections show normal architecture of glomerulus, Bowman's capsule, the convoluted tubules, and distal tubules (Figure 13A). The arsenic treated section of kidney showed deshaped glomerulus and Bowman's capsule. Morever, severe haemorrhage in the kidney tissue were observed denotes the abnormal filtration process in the kidney due to arsenic induced toxicity. (Figure 13B). But, after administration of honey there was significant amelioration in the nephrocytes, especially in the glomerulus, Bowman's capsule and convoluted tubules, denotes the normal functioning of the nephrocytes (Figure 13C).

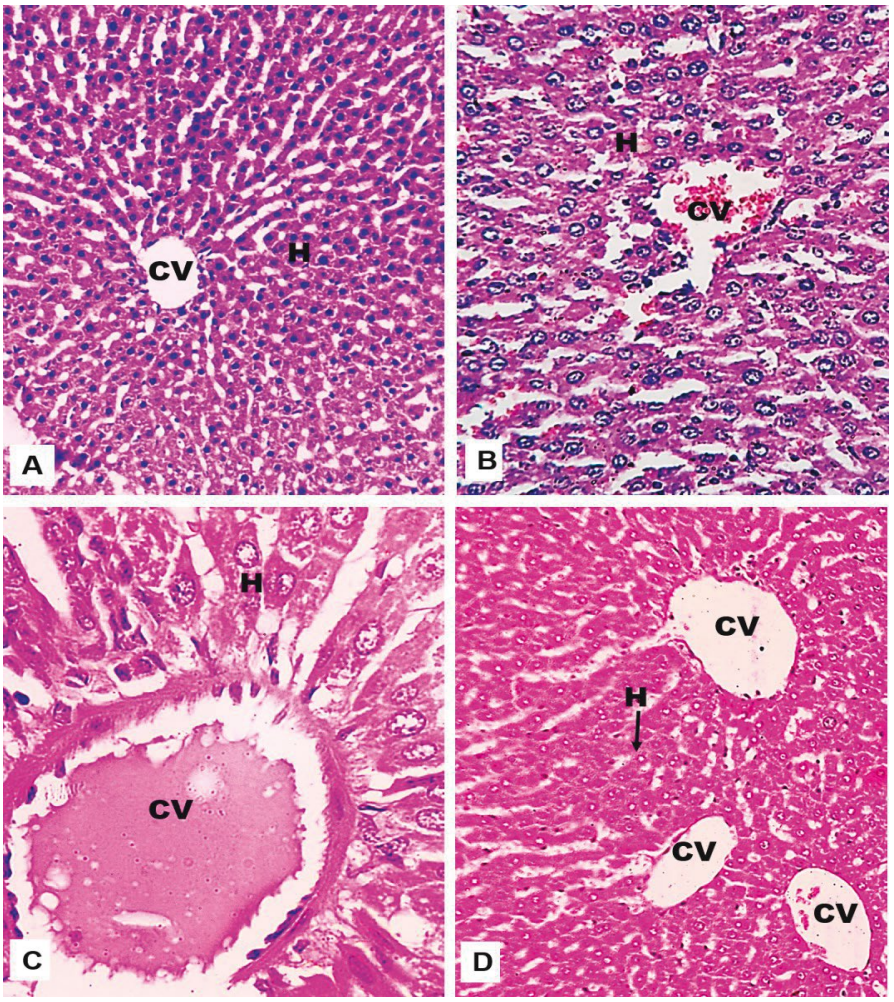

Figure 12. Microphotograph sections of liver of rat stained with haematoxylin and eosin (H&E × 500). **[A]** Showing control rat liver with normal architecture of hepatocytes (H), central vein (CV), with sinusoids. The hepatocytes are well arranged in the sinusoids **[B]**. Showing arsenic treated rat liver sections with degenerated hepatocytes (H) with central vein (CV). Pyknotic nuclei of the hepatocytes are also clearly visualized. The increased number of Kupffer cells (pin shaped) can be seen in the tissue denotes the degree of inflammation in the tissue. Hemorrhage in the sinusoidal spaces can also be observed. **[C&D]** After the administration with honey, the rat liver sections show significant normalization in the hepatocytes (H) with central vein (CV).

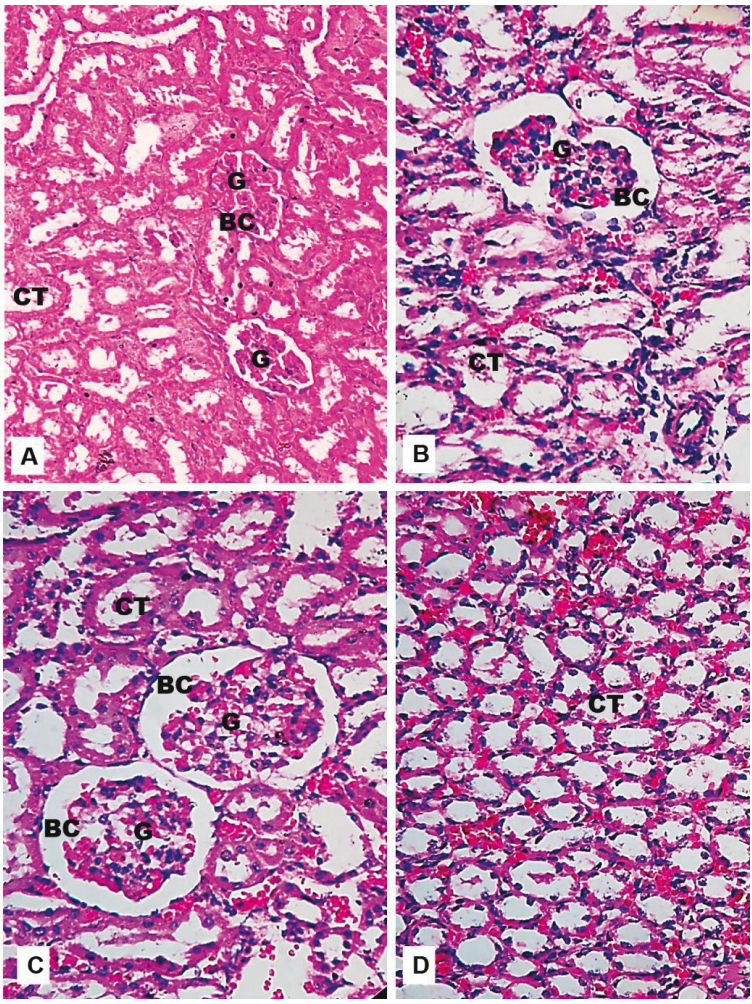

Figure 13. Microphotograph sections of kidney of rat stained with haematoxylin and eosin (H&E × 500). **[A]** The control rat kidney sections show normal architecture of glomerulus (G) with Bowman's capsules (BC). The endothelial cells of convoluted tubules are also in normal architecture. **[B]** The arsenic treated rat kidney sections show significant degeneration in the glomerulus (G) and Bowman's capsule (G) with haemorrhage in the entire kidney tissue. The convoluted tubules (CT) are also severely damaged. **[C&D]** The honey treated rat kidney section shows significant amelioration in the nephrocytes especially the glomerulus (G), Bowman's capsule (BC) and convoluted tubules (CT) denotes the normal functioning of the kidney tissue.

Discussion

Arsenic ingestion by gastrointestinal tract reaches the blood and other vital organs of the body, which in turn reflects its impact on the organ system and to the entire body of the animals. In the present study, in the arsenic treated rats, there was significant ($p<0.005$) decrease in the haematological parameters such as RBC counts, WBC counts and haemoglobin percentage in comparison to the control group of rats. This denotes that the arsenic toxicity has caused damaged the hematopoietic stem cells of the rats by which there is significant changes observed in the haematological parameters of the rats. But, after the administration of honey to the arsenic pre-treated rats, there was significant normalization in the RBC counts, WBC counts and haemoglobin percentage denotes the amelioration at the haematological level. Biochemical parameters are second level of the indicators which reflects the damage at the biochemical level in the tissue level. The liver and the kidney function tests are the important enzyme markers of the body which provide the significant information related to the toxicity in the body. In the present study, there was significant increase ($p<0.005$) in the SGPT, SGOT, alkaline phosphatase, bilirubin, urea, uric acid and creatinine levels, denotes that arsenic toxicity causes significant damage to these vital organs. But, after the administration with honey in arsenic pre-treated rats, there was significant restoration in these liver functions and kidney function tests levels. The third level of toxicity assessment is observed through the histopathological studies. In the present study, the histopathological study of liver and kidney showed severe damage in the liver and kidney tissues. In the arsenic treated liver tissue sections there was significant damage observed in the hepatocytes, central vein, portal vein, and sinusoidal spaces. The increase in the number of Kupffer cells denotes that arsenic toxicity has caused severe inflammation in the liver cells. Apart from this, there was haemorrhage in the sinusoidal spaces. While in kidney tissue the damage was caused to glomerulus, Bowman's capsules, convoluted tubules and ductal tubules. The degeneration caused due to arsenic toxicity has led to haemorrhage in the kidney tissue has hampered the glomerular filtration process. But, after the administration of honey to the arsenic pretreated rats, there was very significant restoration observed in the liver and kidney tissues observed. The hepatocytes, central vein, sinusoidal spaces all have significantly restored in comparison to the arsenic treated rat liver. Similarly, in kidney tissue also there was significant restoration in the nephrocytes especially the glomerulus, Bowman's capsule, convoluted tubules, distal tubules. This denotes that honey has antidote properties, which can control the damage caused by the arsenic induced toxicity. The entire restoration in the organ level observed is due to the honey's rejuvenating properties, antioxidant properties which has combat the damage caused by the arsenic induced toxicity [27-43, 53].

The defense mechanism at the cellular level is maintained by the oxidant- antioxidant system. In the present study, there was significantly very high lipid peroxidation levels observed in the arsenic treated group of rats in comparison to the control group of rats denotes the failure of the defense mechanism in this group. But, after the administration with honey, there was significant restoration in the lipid peroxidation levels, denotes that honey possesses antioxidant properties [24-26, 54-60].

The honey possesses active ingredients such as polyphenols which played the vital role in the normalization in the cellular functions in the arsenic induced toxicity. They rejuvenate the damage caused by arsenic through the antioxidant mechanism normalizing the body functions at the haematological levels, biochemical levels and histopathological level [61-64].

Conclusion

From the entire study it can be concluded that honey possesses antioxidant properties, which has antidote effect against arsenic induced toxicity in rats. The therapeutic effect of honey can be used as antidote drug against the arsenic induced toxicity.

Author contributions

The entire experimental work was conceptualized by M.K., S.S. and A.K. The manuscript's principal author M.K. contributed the majority of writing activities, but support was also provided by S.S, and A.K., Literature search was done by M.K. Figures were developed by M.K. and A.K. The experimentation and data analysis were carried out by M.K. The figures were designed by M.K. and A.K. The statistics and data interpretation were done by M.K. The final manuscript writing was done by M.K. S.S. and A.K. All authors read and approved the final manuscript.

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Conflicts of interests

The authors declare that they have no conflicts of interest.

Ethics approval

Before the start of the experiment, ethical approval of the experimental work was obtained from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, Government of India (CPCSEA Registration no. IAEC No.1129/PO//ReBi/S/07/CPCSA). This research work was approved from the animal house of Institutional Animal Ethics Committee (IAEC) with IAEC No. IAEC No. 2021/1E-06/10/21.

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