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Ameliorative effect of G*anoderma lucidum* on sodium arsenite induced toxicity in Charles Foster rats

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Abstract

Groundwater contamination in India exposes an estimated 70 million people to arsenic. Over 10 million people in the state of Bihar (India) are at risk of arsenic poisoning. As reported 27 districts of the state's thirty-eight districts are under a state of catastrophe. Symptoms noticed by those who have been exposed to the arsenic caused disease are- lack of appetite, neurobehavioral problems, hyperkeratosis, and melanosis on the skin. So, this study's primary objective is to find novel approaches to treat arsenic poisoning in rats using the Charles Foster model.

Following permission from the Institutional Animal Ethics Committee, the animals were divided into three groups: one group served as a control, the second group received arsenic treatment, and the third group received Ganoderma lucidum extract after arsenic treatment. For the arsenic group, the rats were given 8 mg/kg body weight of sodium arsenite orally every day for 90 days, and then for 60 days, they were given 80 mg/Kg body weight of *Ganoderma lucidum* extract via gavage. Their biochemical values, including those of the liver and kidneys, were found to be increased. In addition, their levels of free radicals, including lipid peroxidation, were measured and found to be substantially higher. In addition, the levels of arsenic in the kidney and liver tissue were very high. However, biochemical and lipid peroxidation levels were significantly restored after administration of *Ganoderma lucidum* ethanolic extract. Arsenic levels in rat liver and kidney tissues were also found to be reduced. *Ganoderma lucidum* has a therapeutic impact against arsenic-induced toxicity,according to the present research.

Keywords: Charles Foster rats, arsenic induced model, biochemical assays, liver and kidney toxicity, Ganoderma lucidum extract, therapeutic effects.

Introduction

Around the globe, pollution levels have risen dramatically in recent decades, with groundwater contamination rising to the forefront as a major concern. People across the globe face serious health hazards due to arsenic-contaminated groundwater. Arsenic poisoning is threatening 300 million people globally; among them, whereas about 70 million in India and 10 million in Bihar's Gangetic plains are at danger. Skin symptoms, neurological problems, hormonal imbalances, gastrointestinal disorders, cardiovascular diseases, etc. were among the many ailments that these individuals had as a consequence of their exposure. There has

been an alarming upsurge of cancer cases in this exposed area. So far, all efforts to resolve the problem have focused on water. The idea of arsenic-free water, however, has greatly reduced the toxicity. Still, arsenic-containing water is being used to cultivate the crops. This indicates that arsenic has entered the human body system via biomagnification, coming from the environment (Shaji et al., 2021; Hassan, 2018; 2019; 2020; Kumar et al., 2022[a]; Richards et al., 2021 & 2020).

Bihar inhabitants are particularly vulnerable to the harmful effects of arsenic poisoning. Major health issues may develop in those who have been drinking water contaminated with arsenic for an extended length of time. Exposed people have shown signs of arsenicosis, which include skin problems, gastrointestinal issues, lung disease, cardiovascular disease, hormone imbalance, lack of hunger, weakened immunity, changes in bowel habits, neurobehavioral abnormalities, and cancer poisoning (Chakraborti et al., 2003 & 2016; Kumar 2022[a], Kumar et al., 2022[b,c]; Kumar et al., 2020[a,b]; Kumar et al., 2021[a,b,c,d]; Kumar et al., 2020; Kumar et al., 2016; Kumar et al., 2015; Rahman 2019[a,b]; Kumar and Ghosh 2021 & 2019[b]).

Hence, to address all of this complexity, a bio-remedial approach is necessary. The whole plant, including its leaves, blossoms, and roots, are used in Ayurvedic treatment. Traditional medicine makes extensive use of the plant extract in the treatment of several diseases and conditions, including as diabetes, malaria, leukemia, and Hodgkin's disease. According to traditional medicine, wasp stings, sore throats, and a newborn eye wash prepared from flower extracts may all be alleviated by gargling with the juice of the leaves. The anti-cancer and anti-tumor actions of the plant are due to the alkaloids contained in its stems and leaves. The hypertension and blood sugar levels may be controlled with the use of the leaves. To some extent, the alkaloids have sedative andrelaxing effects. In Asia, the fungus *Ganoderma lucidum* (*G. lucidum*, Reishi) has a long history of use as a medicine to promote wellness and cure a wide range of illnesses. Triterpenes and polysaccharides, two of *G.lucidum's* rich chemical components, have a wide range of biological functions, such as preventing oxidation, inflammation, liver diseases, tumor development and metastasis, and more. A number of recent studies have shed light on the potential therapeutic effects of *G. lucidum* and its extracts on a variety of kidney disease (CKD) and acute kidney injury (AKI) pathogenesis, such as diabetic nephropathy, renal ischemia reperfusion injury, chronic proteinuric renal diseases, cisplatin- induced renal injury, adriamycin-induced nephropathy, and many more. Clinical studies have alsoshown that *G.lucidum* has strong bioactivities that combat renal illness (Geng et al., 2020 & 2019; Bishop et al., 2015; Batra et al., 2013). Therefore, the present research aims to determine the ameliorative effect of *G. lucidum* on arsenic-induced toxicity in Charles Foster rats.

Materials and Methods

Ethics approval: Approval for this research was given by the Institutional Animal Ethics Committee of Mahavir Cancer Sansthan and Research Centre at Phulwarisharif, Patna, Bihar, India.

Animals: Male Charles Foster rats weighing an average of 180g were utilized in this investigation. The animals had adequate housing, including group cages with food and water *ad libidum*.

Chemical: Sodium arsenite, a form of arsenic purchased from Merck-Sigma Aldrich, was used in the research. An oral dose of 8mg/Kg of body weight was made for the animals after dosage titration.

Medicinal Plant Used: In the study, the medicinal plant utilized was *Ganoderma lucidum* (a mushroom) which was procured from the local market of Patna, Bihar, India, and later recognized by a Botanist at A.N. College, Patna, Bihar, India, as an antidote for arsenic-induced poisoning in rats. The mushroom was dried in the oven for 48 hours. After being soaked in alcohol for 48 hours, were finally ready to use. To obtain the ethanolic extract, the powdered mushroom was run through a vacuum evaporator. The titration resulted in a reference dosage of 80 mg/Kg.

Experimental design: Animals were divided into 03 groups - Group-I – Control group (n=6), Group-II– Arsenic treated group (n=12), Group-III – *G. lucidum* extract treated group (n=6). The control group received only plain water and food to eat. The arsenic treated group were given sodium arsenite at the dose of 8 mg/Kg body weight per day for 90 days. The arsenic-pretreated rats (treated with arsenic for 90 days) received *G. lucidum* extract at a dosage of 80 mg/Kg body weight each day for 60 days. At the completion of the experiment, all of the rats were sacrificed, blood was drawn for haematological and biochemical analysis, and important organs including theliver and kidney were removed for histological analysis.

Haematological study: The blood samples were analyzed according to the standard techniques for haematological parameters including hemoglobin percentage, platelet counts, red blood cell counts,and white blood cell counts.

Biochemical assays: Centrifugation was used for 15 minutes at 3000 rpm to separate serum from the obtained blood samples. The serum that was collected was then used in biochemical tests that assessed kidney and liver function. Using a Spectrophotometer (UV - Vis) (UV-10, Thermo Fisher, USA), the biochemical analysis was conducted following the standard kit technique (Coral crest). In this study, biochemical parameters were measured using thefollowing methods: liver function tests (SGPT and SGOT) were estimated according to (Reitman & Frankel, 1957), total bilirubin activity, alkaline phosphatase (ALP) assay and the method of(Kind & King, 1954) for the Alkaline Phosphate experiment. Urine (Fawcett 1960, Berthelot 1859),creatinine (Toro and Ackermann 1975), and uric acid (Bones and Tausky 1945) were the kidneyfunction tests (KFT) that were used. (Draper and Hadley, 1992) was the methodology used to conduct the lipid peroxidation investigation.

Histopathological study: For the histopathological study, the procedure was used to fix the tissue samples in 10% neutral formalin for a minimum of 24 hours. Following a gradient of increasing ethanol concentrations, the tissues were prepared for embedding in paraffin wax blocks. Microscopic slides were prepared by trimming thin (5 mm) sections of paraffin blocks, staining them with Delafield's haematoxylin and Eosin Y, and then subjecting them to various concentrations of alcohol. The stained slides were examined under a microscope for histopathological examination (Cardiff et al., 2014).

Statistical analysis: The statistical analysis was conducted using GraphPad 5.0 to do one-way ANOVA testing. The importance of all other factors was assessed using Dunnett's approach.

Results

Haematological study: Compared to the control group rats, the rats treated with arsenic had significantly lower red blood cell, white blood cell, platelet, and hemoglobin percentage counts. However, when treated with *G.lucidum* extract, all of these parameters weresignificantly normalized ($p<0.05$) (Table 1).

Table 1.: Rats of several treatment types were tested for various haematological markers. Statistics are presented as Mean ± SE. (One way ANOVA Test in various group of rats (n=6))

Group	Control	90 Days arsenic treated	60 Days *G. lucidum* treated
RBC ($\times10^6$/mm^3)	6.23 ± 1.65	1.96 ± 2.55	5.14 ± 4.85
HGB (g/dL)	13.98 ± 3.79	5.69 ± 1.45	13.55 ± 3.78
HCT (%)	42.28 ± 3.88	16.8 ± 1.92	40.89 ± 5.45
MCV (fL)	67.9 ± 5.82	85.7 ± 8.45	79.6 ± 9.74
MCH (pg)	22.4 ± 3.28	29.0 ± 3.04	26.4 ± 5.04
MCHC (g/dL)	33.1 ± 1.09	33.9 ± 3.78	33.1 ± 3.95
WBC ($\times10^3$/mm^3)	7.24 ± 4.44	17.94 ± 6.43	9.65± 3.07
Platelets ($\times10^3$/mm^3)	330 ± 16.2	84 ± 34.2	245 ± 11.55

Biochemical Study:

1) **SGPT Assay:** The SGPT levels were significantly higher in the rats that were arsenic- treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal SGPT levels (Figure 1).

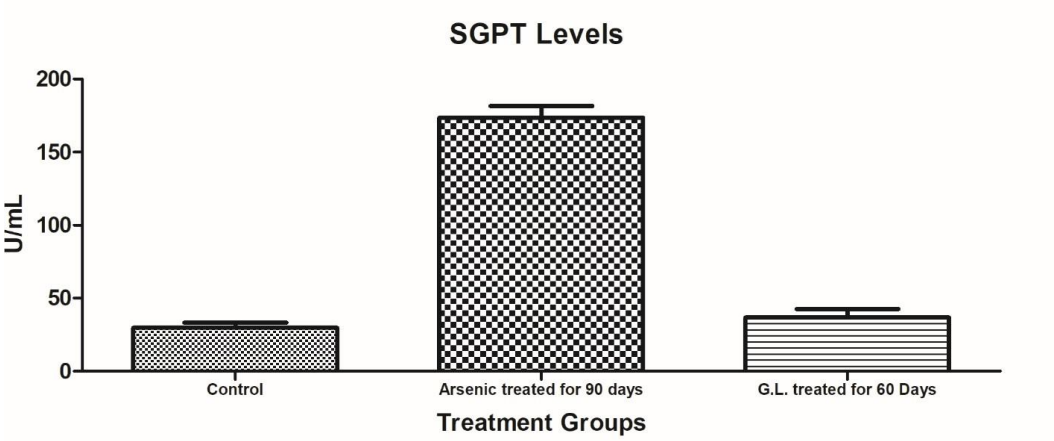

Figure 1. SGPT levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

2) SGOT Assay: The SGOT levels were significantly higher in the rats that were arsenic- treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal SGOT levels (Figure 2).

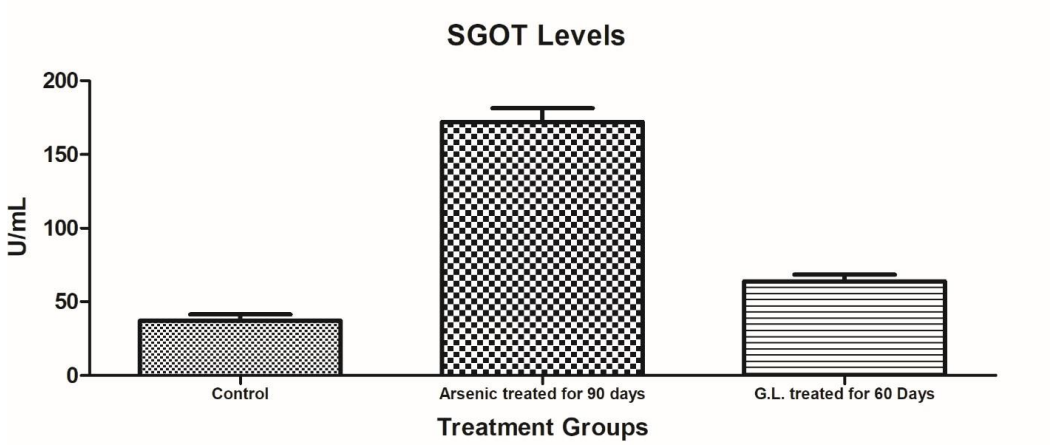

Figure 2. SGOT levels of the treated groups (One way ANOVA Test in various group of rats (n=6, values displayed as Mean ± SE)

3) Alkaline Phosphatase (ALP) Assay: The ALP levels were significantly higher in the rats that were arsenic-treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal ALP levels (Figure 3).

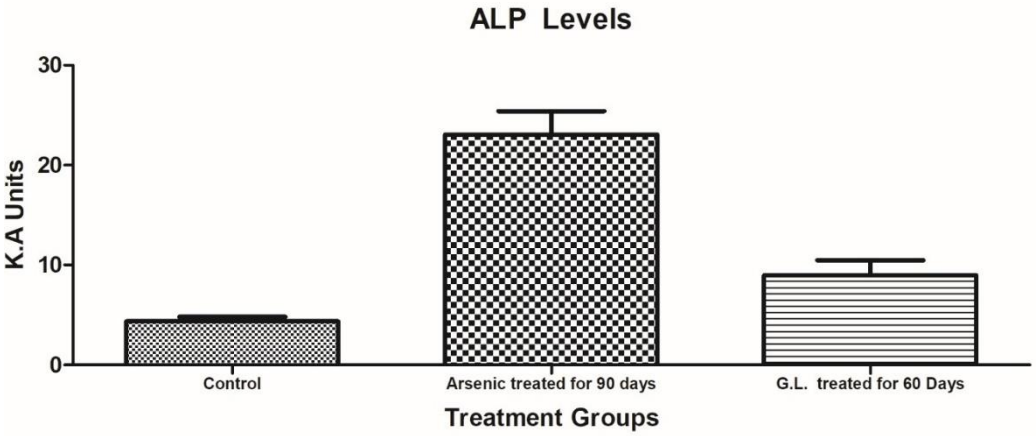

Figure 3. Alkaline phosphatase levels of the treated groups (One way ANOVA Test in various group of rats (n=6) values displayed as Mean ± SE)

4) **Bilirubin Assay:** The bilirubin levels were significantly higher in the rats that were arsenic- treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal bilirubin levels. (Figure 4).

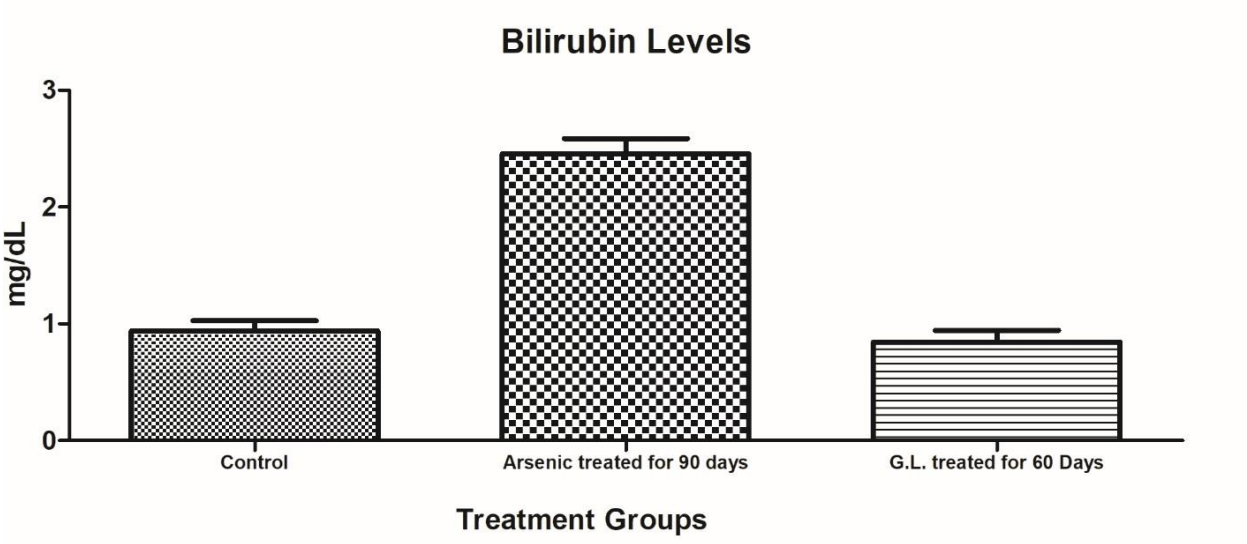

Figure 4. Bilirubin levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

5) **Urea Assay:** The urea levels were significantly higher in the rats that were arsenic-treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal urea levels (Figure 5).

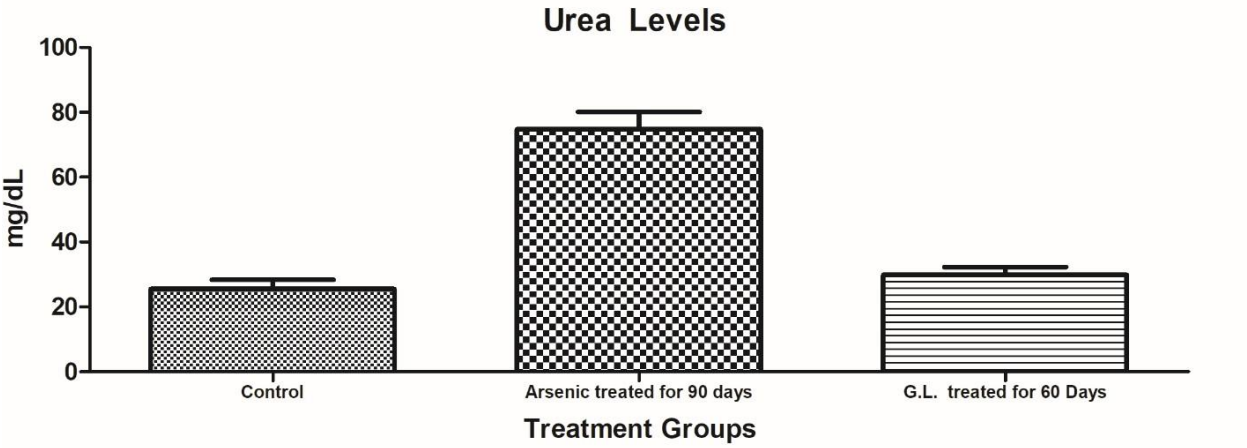

Figure 5. Urea levels of the treated groups (One way ANOVA Test in various group of rats (n=6), values displayed as Mean ± SE)

6) **Uric Acid Assay:** The uric acid was significantly higher in the rats that were arsenic-treatedcompared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal uric acid levels (Figure 6).

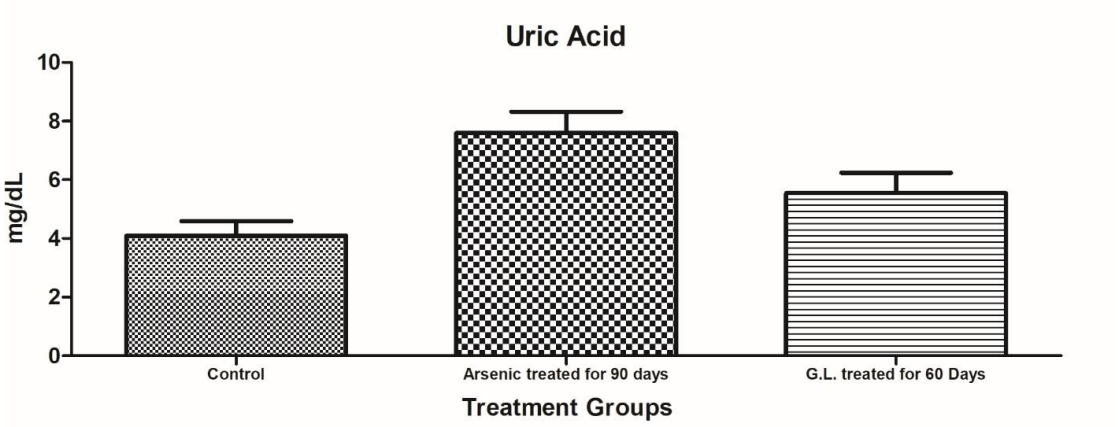

Figure 6. Uric acid levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

7) Creatinine Assay: The creatinine levels were significantly higher in the rats that were arsenic-treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal creatinine levels (Figure 7).

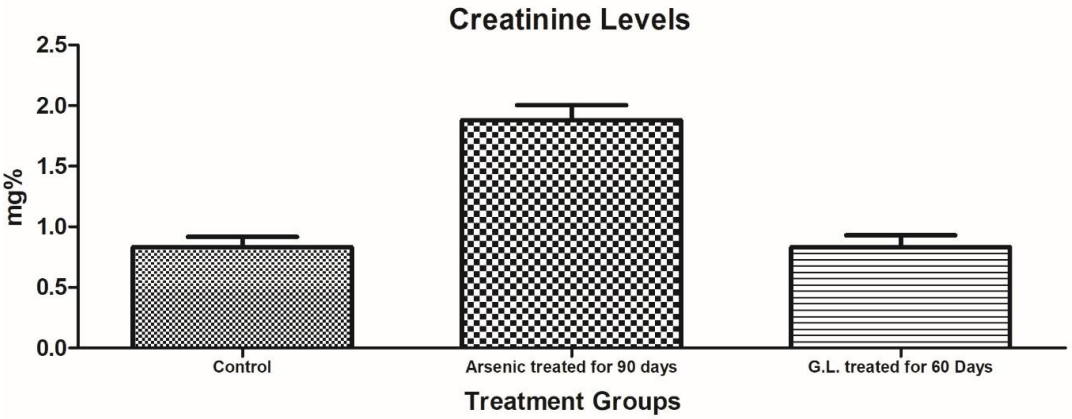

Figure 7. Creatinine levels of the treated groups (One way ANOVA Test in various group of rats, (n=6) values displayed as Mean ± SE)

8) Lipid Peroxidation (LPO) Assay: The LPO levels were significantly higher in the rats thatwere arsenic-treated compared to the control rats ($p<0.05$). After rats were exposed to arsenic, treatment with *G.lucidum* extract significantly ($p<0.05$) restored back normal LPO levels (Figure 8).

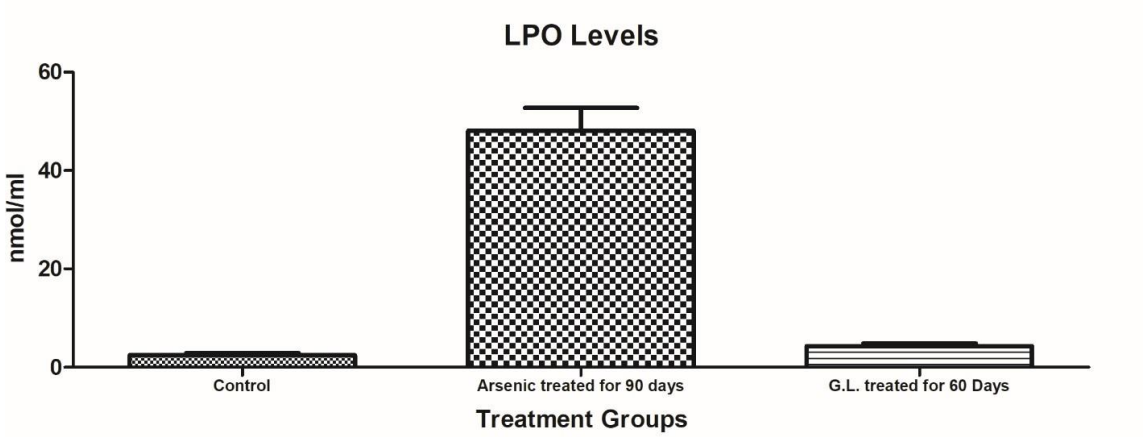

Figure 8. Lipid peroxidation levels of the treated groups (One way ANOVA Test in various groupof rats, (n=6) values displayed as Mean ± SE)

Histopathological Study:

Figure 9A depicts a microphotograph section of the liver, which according to the present research demonstrates normal architecture of the liver with hepatocytes appropriately distributed in the sinusoids, which indicates that the liver cells are operating normally. The liver section of the rat that was exposed to arsenic had a high level of cellular synthesis, with many apparent ruptures in the endothelial cells lining the central veins. A rise in the Kupffer cell counts is indicative of enhanced macrophagic activity within the liver's cell population. In addition, the liver tissue very clearly displays hemorrhages in the sinusoids. **Figure 9B** shows that this indicates that the liver cells are not operating properly. But after 60 days of treatment with *G.lucidum* extract, the hepatocytes, central vein, and sinusoids showed signs of substantial restoration. The arrangement of hepatocytesin the sinusoids is proper, and liver function seems to be normal. In addition, as seen in **Figure 9C,**the liver is functioning appropriately as there are no Kupffer cells. **Figure 9D** shows that the kidneyhistological sections show typical glomerulus, Bowman's capsule, convoluted tubules, and distal tubules. A distorted glomerulus and Bowman's capsule with hemorrhage are shown in the arsenic-treated kidney section. In addition, significant hemorrhage inside the kidneys might be seen, whichcould be a sign of an abnormal kidney filtration mechanism brought on by arsenic poisoning **(Figure 9E). Figure 9F** shows that nephrocytes, especially those in the glomerulus, Bowman's capsule, and convoluted tubules, showed a substantial reduction after treatment with *G.lucidum* extract, suggesting normal nephrocytic activity.

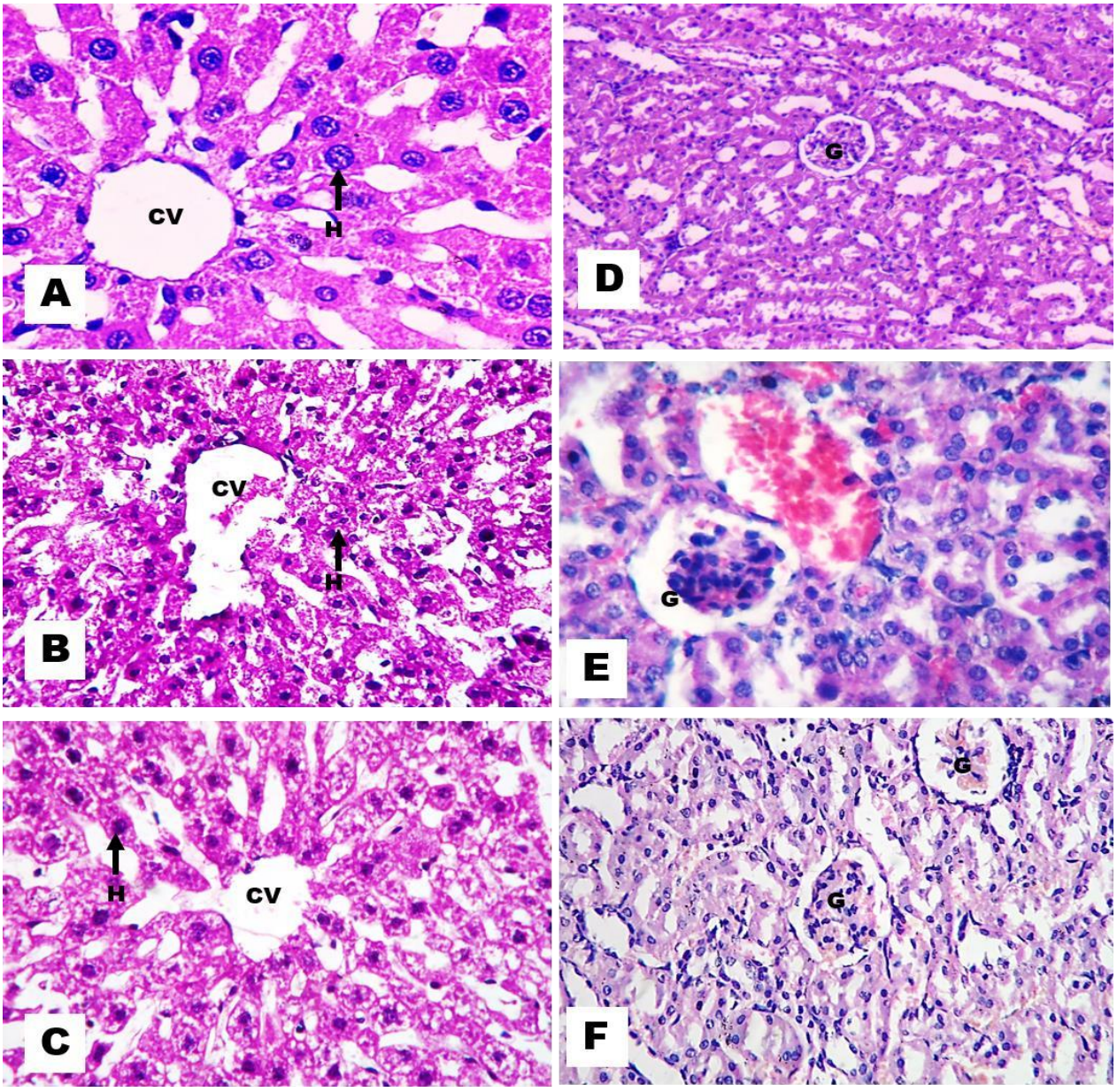

Figure 9: [A] Haematoxylin and eosin-stained sections of rat liver (H&E 500) were microphotographed. The sinusoidal structure, central vein (CV), and hepatocyte (H) are all clearly visible and functional in the liver of a healthy control rat. **[B]** In the sections of liver which was treated with arsenic showed sinusoids well-structured with hepatocytes. The pyknotic nuclei in thehepatocytes (H) and the central vein (CV) in rat liver and moreover the number of Kupffer cells, which are pin-shaped cells, depicted severity of tissue inflammation. Additionally, there was hemorrhage in the sinusoidal spaces. **[C].** But after the administration with *G.lucidum* in hepatocytes (H) with central vein (CV) significantly normalized. **[D]** Rat kidney sections stained with H&E 500 (haematoxylin and eosin) reveal Normal glomerulus (G) and Bowman's capsules (B) shown in sections of normal rat kidneys. **[E].** In addition, the endothelial cells that line the kidney's tubules are an essential component. Arsenic causes severe hemorrhage and glomerulus (G) and Bowman's capsule degeneration in rat kidney tissue. In addition, the convoluted tubules (CT) have suffered extensive damage. **[F].** The glomerulus (G), Bowman's capsule (B), and convoluted tubules (CT) all return to normal after being treated with the *G.lucidum.* Thenephrocytes in this section of the rat kidney demonstrate substantial recovery.

Mean ± S.D. values are used to indicate the quantified histological damage score in liver tissue of ratsthat were either treated or not treated (n=6). In order to measure hepatocyte degeneration, 100 hepaticcells were counted in each rat. For the remainder of the histopathological alterations, we randomly examined 20 microscopic areas using X40 and H&E. (Table 2.).

Table 2: Quantified scoring of histopathological damage in the liver tissue

Group	Control	90 Days arsenic treated	60 Days *G.lucidum* treated
Degenerated hepatocytes	0.42 ± 0.54	53.88 ± 2.67	20.19 ± 1.92
Vacuolization	0.51 ± 0.32	22.84 ± 3.93	9.75 ± 3.04
Haemorrhage	0.12 ± 0.23	18.18 ± 2.19	5.17± 2.05
Central vein degeneration	0.76 ± 0.11	33.96 ± 5.88	7.34 ± 1.19
Portal vein degeneration	0.24 ± 0.03	20.33 ± 4.86	6.49 ± 2.09

Mean ± S.D. values are used to indicate the quantified histological damage score in kidney tissue of rats that were either treated or not treated (n=6). To determine the extent of tubular degeneration in each rat, 100 tubules were counted and randomly examined 20 microscopic areas for histopathological alterations (X40; H&E) (Table 3.).

Table 3: Quantified scoring of histopathological damage in kidney tissue

Group	Control	90 Days arsenic treated	60 Days *G.lucidum* treated
Tubular degeneration	0.23 ± 0.38	78.34± 8.85	15.18 ± 2.94
Glomerulus degeneration	0.83 ± 2.67	23.93 ± 3.64	11.32 ± 1.04
Haemorrhage	0.34 ± 0.73	19.92 ± 1.05	2.72 ± 1.93
BC membranedegeneration	0.29 ± 0.19	29.46 ± 2.67	5.15 ± 2.03
Vacuolization	0.18 ± 0.21	29.17 ± 4.05	9.42 ± 2.66

Discussion:

Consumption of arsenic-contaminated groundwater is associated with increased risk of neurological and cardiovascular diseases. Since arsenic+3 is more hazardous than arsenic+5, bonding to the sulfhydryl group and creating methylation of it enhances the likelihood of arsenic causing metabolicmalfunction (Yamanaka, 2004).

The rat model exposed to arsenic showed notable changes in the parameters studied. In the haematological study, significant decreases were seen in white blood cell count, platelet count, and RBC indices variables such as haemoglobin percentage, Hct percentage, and MCHC. This shows that the myelosuppression-induced breakdown of the defense system was caused by the very high arsenic levels. The biochemical measures showed a notable rise in the levels of bilirubin, SGPT, SGOT, ALP, bilirubin, urea, uric acid, and

creatinine. Lipid peroxidation levels rise in response to changes in cellular oxidative activity. An extended period of exposure to arsenic causes the animal's body to deteriorate and die. Consistent with the histological criteria, arsenic-exposed animals exhibited comparable degradation in the central vein, sinusoids, and hepatocytes as compared to the control group. Kupffercell proliferation indicates that the liver's defenses have failed. In contrast to the control group, kidneytissue showed substantial damage to nephrocytes, especially in the glomerulus, Bowman's capsule, convoluted tubules, and ductal tubules.

Because of its very short half-life, arsenic usually enters the body via the digestive tract and passes to the circulatory system, where it remains for the shortest possible duration (Lu et al., 2007). The majority of arsenic is excreted in urine and bile. According to Helleday et al. (2000), the inhibition of pyruvate dehydrogenase by arsenic's As+3 form results in cellular energy loss. When the body's defense systems are disorganized, causing cells to be damaged and eventually die, cells generate apoptosis inducing factors (AIF). When the cellular apoptotic system does not activate, carcinogenesis in vital organs such the liver, kidneys, bladder, etc., takes place (Mallikarjuna et al., 2003; Yamanaka et al., 2004). Many studies have shown that methylated arsenic, such as DMA+5, stimulates tumor development and has detrimental consequences (Yamamoto et. al., 1995; Wanibuchi et. al., 1996; Cohen et al., 2006 & Cohen 2014; Kumar et al., 2023a,b). Soni et al. (1993) found that arsenic builds up in the body over time, particularly in the kidneys and liver. Since arsenic interacts directly with glucocorticoid receptors (GRs), it blocks GR-mediated transcription. Aneuploidy, micronucleus generation, DNA-protein cross linking, and sister chromatid exchange are chromosomal damage and mutations that result from spindle apparatus disruption (Miller et al., 2002; Huang et al., 2004; Duker et al., 2005). Recent studies have shown that arsenic may cause severe chronic kidney disease. The researchers found that arsenic lowers the glomerular filtration rate and increases urine albumin secretion (Chen et al., 2011; Hsueh et al., 2009; Zheng et al., 2013 & 2014; Jha et al., 2013). According to many studies (Styblo et al., 2002; Carter et al., 2003; Yousefsani et al., 2018; Zhang et al., 2014), DMAV causes harm to the nephrocytes when it is exported from the liver to the kidney. The present study found substantial biochemical and cellular changes in kidney and liver tissues, lending credenceto the previously stated theories. The present study confirms previous findings that the kidneys and liver play a major role in the elimination of arsenic from the body.

In addition, the antitoxic and antidote action of *G.lucidum* was significantly ameliorated. The result is consistent with the findings of related research on various hazardous models. The *G.lucidum* mushroom was used as an antidote against arsenic toxicity in the current study, and it was found to significantly restore all of the parameters examined, including haematological, biochemical, and lipid peroxidation levels. The cellular level was also significantly restored, with both hepatocyte and nephrocytic functions returning to normal. The active ingredients of *G.lucidum* are triterpenes, polysaccharides, and peptidoglycans are the 3 major physiologically active constituents in *Ganoderma lucidum* which had played vital role in controlling the toxicity caused due to arsenic poisoning (Chen et al., 2024; Liu et al., 2024; Ahmad et al., 2024; Boh et al., 2007; Adamec et al., 2009; Martínez-Montemayor et al., 2019; Unlu et al., 2016; Seweryn et al., 2021; Milosavljevic & Barnes, 2023; Chan et al, 2021). Various studies on other medicinal plants on animal models have been carried out which show significant amelioration against arsenic (Kumar et al., 2022[e]; Kumar et al., 2022[d], Kumar et al., 2020[b]; Kumar 2015[a,b]).

Conclusion

Arsenic causes extensive damage in rats on several levels of study, including the haematological, biochemical, and histological. Nevertheless, the administration of *Ganoderma lucidum* extract resulted in substantial cellular healing. The liver and kidneys' enzyme functions restored back to normal, and there was an apparent amelioration to cellular normality, so the tissues were functioning normal again. This data demonstrates that *G.lucidum* extract mitigates arsenic toxicity to the liver and kidneys. Hence, it possesses the potential to be of a novel, safe anti-arsenic drug.

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Author contributions

M.K., R.K., and A.K. conceived of the whole experimental effort. M.K., the manuscript's primary author, did the bulk of the writing, with assistance from R.K. and A.K. M.K. conducted the literature review. M.K. and A.K. designed the graphical sections. M.K. conducted the experiments and analyzed the results. M.K. and A.K. designed the figures. M.K. analyzed the data and drew the necessary conclusions. M.K., A.K. and R.K. wrote the final draft of the text. Each contributor has reviewed the final version of the paper.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Consent for publish

All the authors provide their consent to publish this article.

Availability of data and materials

All the data on request is available.

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