

Research Article


Ameliorative effect of *Panax quinquefolius* on sodium arsenite induced toxicity in Charles Foster rats

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Article Info

<https://doi.org/10.31018/jans.v14i4.3957>

Received: August 17, 2022

Revised: December 9, 2022

Accepted: December 15, 2022

How to Cite

Kumari, S. *et al.* (2022). Ameliorative effect of *Panax quinquefolius* on sodium arsenite induced toxicity in Charles Foster rats. *Journal of Applied and Natural Science*, 14(4), 1532 - 1541. <https://doi.org/10.31018/jans.v14i4.3957>

Abstract

An estimated 70 million population are exposed to arsenic poisoning in India in the 2020. The present study is aimed to develop the antidote for arsenic-induced toxicity in Charles Foster rats. A total of n=18 rats (12 weeks old) of an average weight of 160 ± 20 g were used for the study. The study group included three groups, n=6 control (Group I: Untreated) and n= 12 (group II) treated with sodium arsenite orally at the dose of 8mg/Kg b.w daily for 6 months. The n= 6 animals were dissected and the rest n=6 (Group III) was administered orally with *Panax quinquefolius* (Ginseng) root ethanolic extract at 300mg/Kg body weight per day for 8 weeks. All the animals were sacrificed after the completion of their respective doses and their blood samples were taken for haematological and biochemical evaluation, while the vital tissues such as liver and kidneys for the histopathological study. The study revealed significant fluctuation ($p < 0.0001/p < 0.001/p < 0.05$) in the haematological parameters viz. leukocyte count, haemoglobin, red blood cell count, haematocrit percentage, MCV, MCH and MCHC and biochemical parameters such as SGPT, SGOT, ALP, bilirubin, urea, uric acid, creatinine and lipid peroxidation in arsenic-treated groups. There was a significant reduction ($p < 0.0001/p < 0.001/p < 0.05$) in the levels of haematological and biochemical parameters after the administration of ginseng extract. Similarly, the histopathological study revealed a high magnitude of degeneration in the hepatocytes and nephrocytes after the treatment of arsenic, but after the administration of ginseng extract, there was significant restoration at the cellular level. Thus, the root extract of *P. quinquefolius* possessed significant ameliorative properties against arsenic-induced toxicity in rats.

Keywords: Arsenic treatment, Ameliorative effect, Charles Foster rats, *Panax quinquefolius*, Root extract

INTRODUCTION

Arsenic poisoning through the groundwater in recent times has become a major health related problem worldwide. An estimated 300 million population is exposed to groundwater arsenic poisoning worldwide with serious health problems. In Asia, an estimated 180 million population are exposed to arsenic poisoning (Shaji *et al.*, 2021; Hassan, 2018). Moreover, in India about 70 million people are exposed to arsenic poisoning. The major chunk of population exposed to groundwater inhabit in the Ganga- Meghna- Brahmaputra plains. India and Bangladesh plains cater the maximum geographical area where arsenic poisoning has caused severe health hazards among the exposed population

(Kumar *et al.*, 2019^a; 2019^b). This has caused disease such as skin manifestations – keratosis in sole and palm, melanosis on body, raindrop pigmentation, loss of appetite, constipation, breathlessness, recurrent cough, cardiovascular problems, hormonal imbalances, gastrointestinal disorders and cancer of skin, lungs, urinary bladder, colon, gallbladder, liver and kidney etc. (Kumar *et al.*, 2020^a; 2021^a; 2021^b; 2021^c; 2021^d). Therefore, there is a need to discover novel drugs which can combat arsenic-induced toxicity in humans. Plethora of medicinal plants have been discovered for its medicinal properties such as immuno-boosters, anti-oxidants, antidotes, hepatoprotective, nephroprotective etc. Few medicinal plants have proven promising effect against arsenic induced toxicity in animal models

(Kumar et al., 2022; 2020^b; Bhattacharya 2017). Ginseng is a medicinal plant widely used for the treatment of various conditions. The pharmacological effects of ginseng have been demonstrated in cancer, diabetes, cardiovascular diseases and have been used for promoting immune function, central nervous system (CNS) function, relieving stress, and for its antioxidant activities (Jung, 1996; Huang et al., 2022; Chen et al., 2022; de Oliveira Zanuso et al., 2022). The major bioactive components of *P. ginseng* are the ginsenosides, a group of saponins with a dammarane triterpenoid structure (Huang 1999). Almost 40 ginsenosides have been isolated from *P. ginseng* root (white and red ginsengs), and novel structures continue to be identified, particularly from *Panax quinquefolius* (American ginseng) and *Panax japonica* (Japanese ginseng) as well as their berries (Attele et al. 2002; Christensen 2009; Wang et al., 2020; Cong et al., 2020; Yang et al., 2022).

Hence, the present study aimed to discover the ameliorative effect of *Panax quinquefolius* root extract against hepatorenal toxicity caused by sodium arsenite in Charles Foster rat model.

MATERIALS AND METHODS

Ethics statement: The entire research work was approved by 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) in the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India. The entire research was approved from the Institutional Animal Ethics Committee (IAEC) with IAEC No. 2015-16/12/2015. Agenda No.3 (c) of the institute.

Animals

Male Charles Foster rats (n=24) of 8 weeks old of weight about 150-180g were procured from the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India which is registered by the CPCSEA, Government of India (CPCSEA Reg. No. 1129/PO/ReBi/S/07/CPCSEA). Before the start of the experiment, the rats were acclimatized for 2 weeks prior to the beginning of the experiment. All the standard measures were taken, such as the laboratory conditions maintained under 12 hours of light and dark cycles and room temperatures maintained at $22 \pm 2^\circ\text{C}$. Animals had free access to food and water *ad libitum*.

Chemicals

Sodium arsenite (98.5%) manufactured by Sigma-Aldrich, USA (CAS Number: 7784-46-5; S7400-100G), Lot# SLBH5736V, PCode 1001683292 was used as a form of arsenic to induce toxicity in animals.

Preparation of *P. quinquefolius* root ethanolic extract

Panax quinquefolius plant roots were procured from the local market of Patna, Bihar and were identified by Dr. Santwana Rani (Botanist), Department of Botany, Patliputra University, Patna, Bihar. The roots of the plant were washed in running water and dried at 37°C in an incubator. The dried roots were subsequently crushed to form a fine powder. They were soaked in absolute alcohol for 48 hours and extracts were separated using Buchi's Rota Vapour. The ethanolic extract dose was calculated after LD₅₀ estimation which was 300 mg/Kg body weight and the final dose calculated was 300 mg/kg body weight for the administration to the sodium arsenite treated rats for 8 weeks (Kumar et al., 2015).

Experimental design

The experimental animals were divided into four major groups each group containing six rats and categorized as follows- Group I: Normal Control group; Group II: Arsenic treated group - Rats were treated orally with sodium arsenite at a dose of 8 mg/kg bodyweight/day for 6 months; Group III: *P. quinquefolius* root extract administered group- Rats were pretreated with sodium arsenite 8mg/kg body weight/day for 6 months followed by administration of *P. quinquefolius* ethanolic extract of root - 300mg/kg body weight/day for 8 weeks. After the entire experimentation, rats were anaesthetized and sacrificed and their blood samples were obtained through the orbital puncture and finally, the serum was segregated for the various haematological and biochemical parameters.

Haematological analysis

RBC counts and WBC counts were determined using Neubauer's chamber by the method of Lewis (1982); Abbey and Belliveau (1978), while for the estimation of haemoglobin, a hemoglobinometer was used using Sahli's method (Wintrobe 1975).

Biochemical analysis

The biochemical analysis was carried out by the standard kit process (coral crest) using the Spectrophotometer (UV - Vis) (UV-10, Thermo Fisher, USA). The biochemical parameter study included the liver function tests- Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) which were estimated by the method (Reitman & Frankel, 1957), Alkaline Phosphate (ALP) by method (Kind and King, 1954), total bilirubin activity by (Jendrassik and Grof, 1938) method while albumin levels were measured by BCG method. The Kidney function test (KFT) were assayed as urea by (Fawcett, 1960 and Berthelot, 1859), creatinine by (Toro and Acker-

mann 1975), and uric acid by (Bones and Taskuy, 1945) method respectively. The TBARS - thiobarbituric acid reactive substances are the marker of lipid peroxidation (LPO). This was evaluated through the double heating method (Draper and Hadley, 1992) 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 was mixed with 2.5 ml of 100gm/L solution of Trichloroacetic acid (TCA) and then centrifuged at 3000rpm for 10 minutes, and then heated in the water bath at 90°C for 15 minutes. After cooling at room temperature, the mixture was further allowed to centrifuge at 3000 rpm for 10 minutes, and 2 ml of the supernatant was mixed with 1ml of 6.7gm/L TBA solution in a test tube which was further heated in water bath at 90°C for 15 minutes and left for cooling at the room temperature. Subsequently, an additional absorbance was measured using UV - visible spectrophotometer (Thermo Scientific UV-10 USA) at 532 nm.

Histopathological study

For the histopathological study, the vital metabolic organs such as liver and kidney tissues were dissected out from all the groups. The dissected tissues were washed in normal physiological saline and then grossed into small pieces and fixed in the 10% formalin fixative for 24 hours. The tissues were then processed through the series of graded alcohol and were embedded into paraffin blocks. The fine sections of about 5µm thickness were cut through the microtome and were stained with hematoxylin and eosin (H&E). For the microscopic observations, the stained slides were viewed under a light microscope (Cardiff et al., 2014).

Statistical analysis

The results are presented as mean ± Standard Deviation (SD) for six rats' individual groups and the total variation represented in a set of data was analyzed through one-way Analysis of Variance (ANOVA). The

differences among mean variance have been analyzed by applying Dunnett's 't' test at 99.9% ($p < 0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

RESULTS

Haematological study

There was a significant decrease ($p < 0.001$) in the haematological parameters - leukocyte count (WBC- 2900/Cu.mm), hemoglobin percentage (6.53 g/dL), red blood cell count (RBC – $2.9 \times 10^6/\text{mm}^3$), haematocrit percentage (19.59 %), MCV (67.55 fL), MCH (22.51 pg) and MCHC in rats exposed to arsenic for six months. But after there was significant ($p < 0.05$) restoration in the hematological parameters after the administration of ethanolic root extract of *P. quinquefolius* - leukocyte count (WBC- 9500/Cu.mm), hemoglobin percentage (12.33 g/dL), red blood cell count (RBC – $4.23 \times 10^6/\text{mm}^3$), haematocrit percentage (36.99 %), MCV (87.44 fL), MCH (29.14 pg) (Table 1).

Biochemical study

After 6 months of arsenic exposure in rats, there was significant ($p < 0.0001$) increase in the biochemical parameters such as in SGPT (127.45 U/ml), SGOT (163.95 U/ml), ALP (25.16 KA Units), bilirubin (2.1 mg/dL), urea (60.22 mg/dL), uric acid (12.3 mg/dL), creatinine (2.85 mg/dL) lipid peroxidation (60 nmol/ml) in comparison to the control group, but there was significant reduction in the levels after the administration of *P. quinquefolius* SGPT (56.42 U/ml), SGOT (52.21 U/ml), ALP (11.35 KA Units), bilirubin (1.2 mg/dL), urea (35.58 mg/dL), uric acid (6.94 mg/dL), creatinine (1.10 mg/dL), LPO (11.43 nmol/ml). (Table 2).

Histopathological study

In the present histopathological study, the normal liver sections (Group-I) showed hepatocytes with normal

Table 1. Changes in the haematological parameters of Charles foster rats exposed to Sodium arsenite and its amelioration by the root extract of *P. quinquefolius*. Data are given as mean ± SD (n=6 each group). The p value ***($p < 0.0001$), ** ($p < 0.001$), * ($p < 0.05$) vs control.

Blood Parameters	Control (Group-I)	Arsenic treated for 6 months (Group-II)	<i>Panax quinquefolius</i> treated for 8 weeks (Group – III)
WBC (/Cu.mm)	7500 ± 12.23	2900 ± 14.42 [*]	9500 ± 4.34 [*]
RBC Counts ($10^6/\text{mm}^3$)	6.23 ± 4.15	2.9 ± 0.85 ^{**}	4.23 ± 1.55 ^{**}
Hb (g/dL)	15.1 ± 1.95	6.53 ± 2.15 ^{***}	12.33 ± 2.05 ^{***}
Haematocrit percentage (Hct) (%)	45.3 ± 2.38	19.59 ± 1.31 [*]	36.99 ± 1.34 ^{***}
MCV (fL)	72.71 ± 13.3	67.55 ± 3.56 [*]	87.44 ± 6.43 ^{**}
MCH (pg)	24.23 ± 2.34	22.51 ± 2.41 ^{**}	29.14 ± 2.01 ^{**}
MCHC (g/dL)	33.3 ± 1.56	33.3 ± 1.32 ^{***}	33.3 ± 0.34 ^{***}

Table 2. Liver and kidney function biochemical parameters activity and lipid peroxidation activity of different treatments in rats. Data are given as mean \pm SD (n=6 each group). The p value ***($p < 0.0001$), **($p < 0.001$), *($p < 0.05$) vs control.

Parameters	Control (Group -I)	Arsenic treated for 6 months (Group -II)	<i>Panax quinquefolius</i> treated for 8 weeks (Group -III)
SGPT (U/mL)	34.23 \pm 3.64	127.45 \pm 3.78*	56.42 \pm 4.23***
SGOT (U/mL)	31.23 \pm 2.49	163.95 \pm 2.34**	52.21 \pm 4.36**
ALP (KA units)	7.24 \pm 1.12	25.16 \pm 3.63**	11.35 \pm 1.27*
Bilirubin (mg/dL)	0.64 \pm 0.25	2.1 \pm 1.12*	1.2 \pm 0.93*
Urea (mg/dL)	30.51 \pm 2.32	60.22 \pm 2.84**	35.58 \pm 2.33*
Uric acid (mg/dL)	5.26 \pm 1.22	12.23 \pm 2.36*	6.94 \pm 1.24**
Creatinine (mg/dL)	0.96 \pm 0.74	2.85 \pm 0.23*	1.10 \pm 0.52**
LPO (nmol/mL)	4.24 \pm 0.36	60 \pm 1.64*	11.43 \pm 1.83*

architecture. The hepatocytes along the central vein were well arranged in the sinusoids denotes the normal cellular function of the liver (Fig, 1A). But, after the treatment with the arsenic (Group-II), there was significant degeneration in the hepatocytes with severe haemorrhage in the central vein. The number of Kupffer cells had also increased many folds signifies the inflammation in the liver tissue (Fig, 1B). However, after the administration of *Panax quinquefolius* (Group-III) there was significant normalization observed in the hepatocytes. The hepatocytes along with the central vein were restored in sinusoids with normal architecture (Fig, 1C). The section of the control kidney (Group-I) showed normal architecture of nephrocytes, Bowman capsule and glomeruli, convoluted tubules and distal tubules (Fig, 2A). But the arsenic treated group (Group-II) showed high degree of damage in the nephrocytes along with the hemorrhages in the glomeruli. The convoluted and distal tubules are also severely damaged (Fig, 2B). However, after the administration of *Panax quinquefolius* (Group-III), there was significant restoration in the architecture of the nephrocytes, with the Bowman capsule and glomeruli, convoluted tubules and distal tubules. However, mild haemorrhages were still persistent (Fig, 2C).

DISCUSSION

In the present study, the arsenic-induced to rats caused serious damage to all the studied parameters level. The gavage arsenic uptake by the rats caused toxicants to reach the vital organs through the blood, where usually the liver metabolizes into smaller compounds – Dimethyl Arsenic Acid (DMA), which is still a carcinogen. It is also eliminated through the kidney after a series of processes (Jha *et al.*, 2013; Styblo *et al.*, 2002; Carter *et al.*, 2003; Tsikas, 2020; Kumar *et al.*, 2019^a; 2019^b; 2015; 2016).

The haematological parameters study showed a signifi-

cant decline in the - leukocyte count (WBC), haemoglobin percentage, red blood cell count (RBC), haematocrit percentage, MCV, MCH and MCHC in rats exposed to arsenic for six months in comparison to the control group rats (Table 1). Similarly, there was a significant ($p < 0.0001$) increase in the biochemical parameters such as in SGPT, SGOT, ALP, urea, uric acid, creatinine, and lipid peroxidation levels ($p < 0.05$) in comparison to the control group (Table 2). Furthermore, at the histopathological level, there was significant degeneration observed at the cellular level in the hepatocytes and nephrocytes after the arsenic induced toxicity. There was an increase in the macrophagic activity (Kupffer cells) denotes the magnitude of the toxicity causing abrupt physiological activity.

Various other studies have also shown that arsenic-induced toxicity in animal models causes severe damage to the body's functions, especially the vital organs of the body leading to abnormal physiological functions. This, furthermore magnified toxicity causes damage at the gene level; hence it is also a genotoxicant (Yamamoto *et al.*, 1995; Wanibuchi, *et al.*, 1996; Cohen, *et al.*, 2006; Kaushal *et al.*, 2019). Long-term arsenic exposure causes metabolic dysfunctions, which leads to cause inflammation in the cells. Chronic inflammation due to continuous exposure leads to a breach in the defense system, which causes inflammatory disease in the organs. They also initiate mutational activity at the gene level, coding wrong proteins and finally damaging the vital functions of the body (Helleday *et al.*, 2000; Mallikarjuna *et al.*, 2003; Yamanaka *et al.*, 2004;; Miller *et al.*, 2002; Huang *et al.*, 2022; Duker *et al.*, 2005; Faita *et al.*, 2013; Roy *et al.*, 2018).

There was significant damage observed in all the studied parameters. In the haematological parameters, the decreased leucocyte counts denote the lowering down of the immune system. This defense breach has led to cause inflammation in the body. The hepatocytes also showed increase in the number of Kupffer cells which

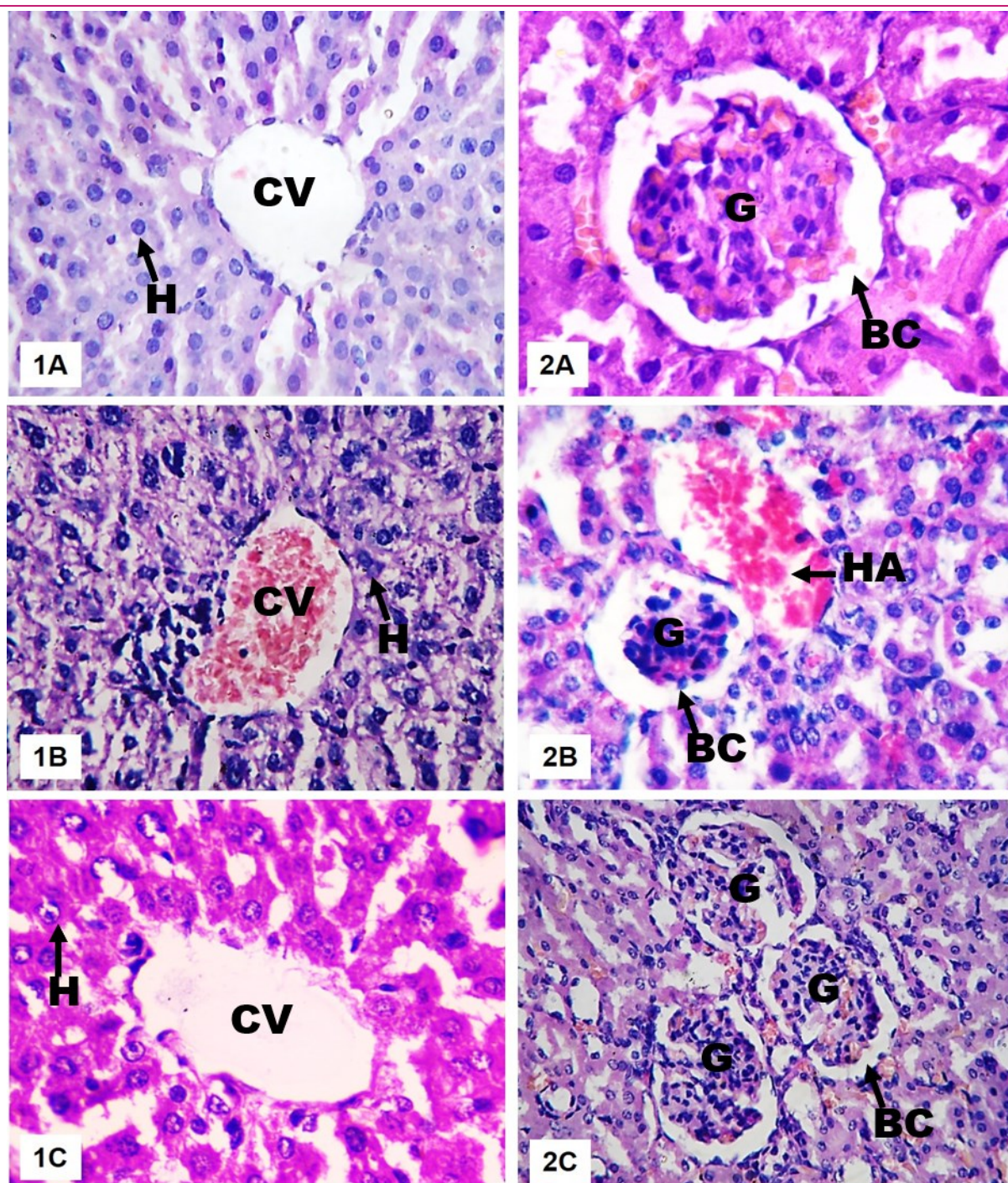


Fig. 1 & 2 (A-C). Group-I- Microphotographic section of liver tissue, hematoxylin and eosin-stained control rats showing the normal architecture of central vein (CV) with hepatocytes (H) with central nucleus with intact dense cytoplasm with well-arranged in sinusoids. X500. 1B Group-II- Rats treated with arsenic for 6 months showing degenerated central vein (CV) with hepatocytes (H) with distorted nucleus and vacuolated cytoplasm. Severe haemorrhage can be clearly seen in the central vein. X500. 1C. Group-III- *P.quinquefolius*, administered to arsenic-treated rats for 6 months for 8 weeks showing restoration in the architecture of central vein (CV) hepatocytes (H); number of Kupffer cells also significantly reduced; sinusoids and the central vein e also significantly restored along with the nuclear material. X500. 2A. Group-I- Microphotographic section of kidney tissue of hematoxylin and eosin-stained control rat showing normal architecture of nephrocytes, Bowman capsule (BC), glomeruli (G), convoluted and distal tubules. X800 2B. Group-II- Rats treated with arsenic for 6 months show high degree of degeneration in nephrocytes (G) along with the haemorrhages (HA) in the Bowman capsule (BC), convoluted and distal tubules X500. 2C. Group-III- In *P. quinquefolius*, administered to arsenic-treated rats for 6 months for 8 weeks, there is significant restoration in the nephrocytes (G) in Bowman's capsule (BC). However, mild haemorrhages are still persistent. X500.

shows that inflammation level is significantly increasing after chronic exposure to arsenic. Similar finding has been observed by other researchers (Tao *et al.*, 2020 in CTSB-inflammasome modulating hepatic stellate cells in arsenic-induced liver fibrosis; Liu and Waalkes 2008 evaluating the effects of malonyl ginsenosides (MGR) on quality control of *Panax ginseng*). This caused the improper functioning of the liver in the arsenic-exposed rats causing fluctuations in the liver function test parameters. Similarly, in the kidney function test, after the arsenic exposure, there was remarkable damage observed nephrocytes – glomerulus and Bowman's capsule. The convoluted and distal tubules endothelial cells are severely damaged as severe haemorrhages were observed in the kidney sections denoting the distorted glomerular filtration rate. Hence the fluctuations in the parameters of the kidney function tests were observed. Similar findings have been observed by other research workers (Chen *et al.*, 2011 in studying the protective effects of *P. ginseng* and its active ingredients against myocardial I/R injury; Hsueh *et al.*, 2009 in the association between urinary arsenic species, plasma lycopene level, and chronic kidney disease (CKD); Zheng *et al.*, 2013 on urine arsenic and prevalent albuminuria & Zheng *et al.*, 2014 on arsenic and chronic kidney disease).

In the present study, *P. quinquefolius* acted as an ameliorating agent against arsenic induced toxicity in rats. Significant amelioration was observed in all the studied parameters, when root extract of this plant at the dose of 300 mg/Kg/ body weight/ per day was administered for 8 weeks. The haematological parameters, the biochemical parameters – liver function tests, kidney function tests and the histopathological study of the liver and kidney showed significant amelioration by the root extract. The primary active ingredient of *P. quinquefolius* is ginsenosides, which have shown a chemopreventive effect. The ginsenosides are a group of natural steroids comprising glycosides and triterpene saponins. There are approximately 40 ginsenoside compounds identified along with the ginsenosides Rb1, Rg1, Rg3, Re, Rd and Rh1 (Sng *et al.*, 2022; Fuzzati 2004; Wu *et al.*, 2018; Liu *et al.*, 2012). Usually, the quantity of ginsenosides found in ginseng species depends upon the type of species used but, the *P. quinquefolius* contains the highest ginsenoside content (Rb1) (Washida and Kitanaka; 2003; Sengupta *et al.*; 2004; Kim *et al.*, 2013). The arsenic long-term exposure causes severe inflammation in the metabolic functioning organs- liver and kidney, which after recurrent mutations in the genes could lead to hepatocarcinogenesis, in which the p53 signaling pathway are hampered. The cyclin D1, cyclin G1 and cell division control protein 2 homolog A prevent the cell cycle from the onset of carcinogenesis. The ginsenoside (Rb1) plays the vital role to downregu-

late the various signaling pathways restricting the uncontrolled cell division (Sherr and Roberts 1995; Basagra *et al.*; 2003; Kwiatkowski 2013; Chen *et al.*, 2021; Loh 2021; Hu *et al.*, 2021). The lipid peroxidation (malondialdehyde (MDA) activity) is the major indicator of the cells showing the level of inflammation. The depletion of the lipids from the membrane due to severe arsenic toxicity denotes a breach in the cellular integrity. But, ginsenoside has the capability to reduce the activity of MDA hence enhancing the membrane integrity (Wang *et al.*, 2016; Fu *et al.*, 2015; Balci *et al.*, 2009; Porfire *et al.*, 2014). The saponins (Ginsenosides) are also found to have an ameliorative role in controlling the arsenic induced toxicity in rats, especially its hepatoprotective and renal protective effect. Similar studies on animals such as mice and rats have been well documented on other toxicological models (Zhang *et al.* (2022) evaluating the effect of total saponins from stems and leaves of *P. quinquefolius* L. ameliorating podophyllotoxin-induced myelosuppression and gastrointestinal toxicity; Ma *et al.* (2017)- evaluating the nephroprotective effects of saponins isolated from the leaves of *Panax quinquefolius* against Cisplatin-induced acute kidney injury; Xu *et al.* (2017) evaluating the effect of saponins isolated from the leaves of *P. quinquefolius* ameliorating the acetaminophen-induced hepatotoxicity in mice).

Conclusion

In the present study, arsenic caused severe damage to the Charles Foster rats at haematological, biochemical and histopathological levels. But, the administration of root extract at the dose of 300 mg/Kg body weight per day significantly ameliorated the arsenic-caused toxicity in rats. The present study thus concluded that *P. quinquefolius* possessed hepatoprotective and nephroprotective effects against arsenic-induced toxicity that may be due to the active ingredient ginsenoside present in it. Hence, it possesses medicinal values against toxicants and can be recommended as a formulation of the novel drug against arsenic.

ACKNOWLEDGEMENTS

The authors are thankful to Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India for providing infrastructural facilities during this work and the P.G. Department of Biotechnology, Magadh University, Bodh Gaya, Bihar, India for providing entire support during the study.

Ethics approval

The entire research work was approved 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) in the animal house of Mahavir Cancer Sansthan and Research Centre, Patna, Bihar, India. The entire research was approved from the Institutional Animal Ethics Committee (IAEC) with IAEC No. 2015-16/12/2015. Agenda No.3 (c) of the institute.

Conflict of interests

The authors declare that they have no conflicts of interest.

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