Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Modulation of Sodium Arsenite-Induced Toxicity in Mice by Ethanolic Seed Extract of *Trigonella foenum graecum*

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Submitted: 20-10-2018 Revised: 01-12-2018 Published: 28-11-2019

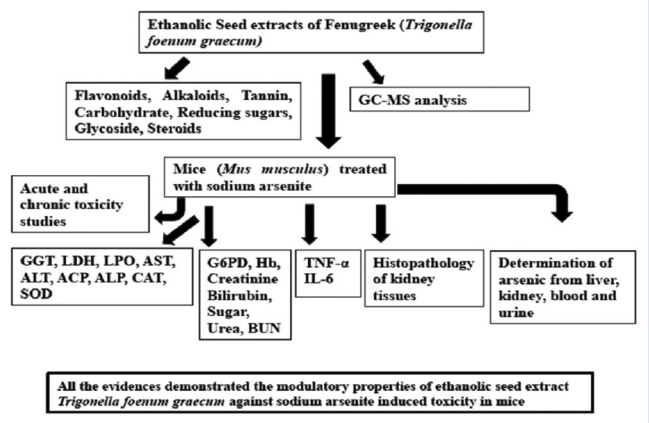
ABSTRACT

Background: *Trigonella foenum graecum* (TG) Linn. (Methi) is widely used as a spice and known for its pharmacological properties. **Objective:** The current study was conducted to examine the efficacy of TG Linn., family: Fabaceae, against sodium arsenite-induced toxicity in mice. **Materials and Methods:** Sixty mice (*Mus musculus*) weighing about 25 g were randomized into six groups; each of ten mice: Group I served as untreated control; Group II received only sodium arsenite (100 ppm) in drinking water for 2 months. The Group III mice fed chronically with sodium arsenite for 2 months as in Group II and then fed a vehicle of 1:20 alcohol to distilled water (1:20) for 15 and 30 days, respectively; Group IV to VI mice were treated as in Group II and then fed with 50, 150, and 250 mg/kg of TG seed extract, once daily for 15 and 30 days. **Results:** The IC_{50} of the seed extract was 66.78 µg/mL, and it reduced the activities of toxicity marker enzymes such as gamma glutamyl transferase, lactate dehydrogenase, lipid peroxidation, aspartate transaminase, alanine aminotransaminase, acid phosphatase, alkaline phosphatase, and pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 ($P < 0.05$ to $P < 0.001$) and elevated the activities of catalase, superoxide dismutase, and G6PD ($P < 0.05$ to $P < 0.001$), a similar trend was also noted with hematological variables. Further, the normal architecture of the kidney was retained in the TG-fed series than arsenic (As)-treated series. Urinary excretion of As was high in treated groups compared to controls ($P < 0.05$ to $P < 0.001$), and 150 mg/kg dose offered better protection than the other two doses. **Conclusion:** TG seed extract has revealed potent antioxidant properties and consequently can be used as a protective agent in As-induced toxicity.

Key words: Antioxidant, biomarkers, methi, modulation, sodium arsenite

SUMMARY

- In the present manuscript, the authors have investigated whether ethanolic seed extracts of *Trigonella foenum graecum* can modulate sodium arsenite-induced toxicity in Swiss albino mice by taking into account several biomarkers of toxicity such as enzymatic and hematological parameters. Further, histological and immunological parameters were also investigated. In addition, we also analyzed phytoconstituents present in the extract which might be responsible for the removal of arsenic from various tissues. The results obtained from the above-stated parameters and also acute and subacute toxicity studies revealed that the extract of the seeds has the potential to be used against sodium arsenite-induced toxicity in mice.



Abbreviations used: TG: *Trigonella foenum graecum*; GC-MS: Gas chromatography-mass spectrometry; TNF: Tumor necrosis factor; IL: Interleukin; DPPH: 1,1-diphenyl-2-picrylhydrazyl; G6PD: Glucose 6 phosphate dehydrogenase; GGT: Gamma glutamyl transferase; LDH: Lactate dehydrogenase; LPO: Lipid peroxidation; AST: Aspartate transaminase; ALT: Alanine aminotransaminase; ACP: Acid phosphatase; ALP: Alkaline phosphatase; CAT: Catalase; SOD: Superoxide dismutase; As: Arsenic; Alc: Alcohol.

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DOI: 10.4103/pm.pm_518_18

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INTRODUCTION

Trigonella foenum graecum Linn. (Fabaceae) (TG), commonly known as fenugreek or "Methi" in vernacular language, is a popular spice in the Indian subcontinent and an important constituent of certain traditional medicines. The leaves of the plant are a rich source of β-carotene, vitamins, iron, and calcium.[1] Chemopreventive properties of Fenugreek seeds have been reported earlier by various investigators.[2,3] Numerous studies such as hypocholesterolemic, antidiabetic, nematicidal, immunomodulatory, antifungal, antibacterial, and strong allelopathic activities of fenugreek extract have also been demonstrated.[4-10] *Trigonella* seeds contain predominantly pyridine alkaloids (choline, trigonelline, and gentianine), flavonoids (quercetin, luteolin, and vitexin), and amino acids (histidine, lysine, and 4-hydroxyleucine), which was reported by other investigators.[11-13] Reduction in oxidative stress in experimental rats and their ability to act as a good galactagogue have also been reported earlier.[14-18] In recent years, there is a growing trend of using

Cite this article as: Biswas SJ, Ghosh G, Dubey VP. Modulation of sodium arsenite-induced Toxicity in mice by ethanolic seed extract of *Trigonella foenum graecum*. Phcog Mag 2019;15:S386-95.

natural compounds from plants and other natural resources, particularly those having known antioxidative properties, in combating various toxicity-related problems and diseases. Therefore, this plant has received considerable attention in recent years, but so far, the extract has not been tested for its possible protective ability against arsenic (As) toxicity, which has already became a menacing problem due to groundwater contamination in parts of India, Bangladesh, and some twenty other countries.

In fact, a high amount of As found in groundwater in deltaic environments is now rapidly spreading in many parts of the world.[19-22] Among the As-affected deltaic environments, the Indo-Gangetic is the worst in terms of human exposure covering a large geographical area. As-related health problems in the Indo-Gangetic plains have been reported by different research groups; however, till date, there is no remedy for treating arsenicosis in the orthodox medical system.[23,24] Several investigations involving plant extract, ayurvedic medicines, and homeopathic drugs have claimed to provide considerable relief against As-induced toxicity.[25-27] Orthodox medicines such as British Anti Lewisite (a dithiol compound), diethylenetriaminepentaacetic acid, and dimercaptosuccinic acid (DMSA) have so far been unsuccessful to treat As-intoxicated patients in view of the harmful side effects of their own. Hence, there is a need for exploring alternative agents which are inexpensive, are easily available, and are affordable by the affected common masses. Thus, the search for suitable plants and their natural compounds is gaining increasing importance in scientific communities, particularly in view of the toxic side effects of most of the synthetic drugs tested so far. In continuation of our earlier works regarding the efficacy of various plant extracts against various types of induced toxicity,[28-30] the present study is designed to evaluate whether the ethanol seed extract of TG can modulate sodium arsenite-induced toxicity in mice.

MATERIALS AND METHODS

Experimental design

The study was conducted on random bred Swiss albino mice weighing about 25 g body weight under the supervision of the Institutional Animal Ethical Committee (1973/GO/Re/S/17/CPCSEA date 19/7/2017, Ministry of Environment and Forest, Government of India). Sixty inbred mice (*Mus musculus*) were randomized primarily into the following groups: Group I consisting of ten mice served as untreated control; Group II consisting of ten mice received only 100 ppm sodium arsenite in drinking water for 2 months. The Group III mice fed chronically with sodium arsenite for 2 months as in Group II and then fed a vehicle of alcohol (Alc) to distilled water (1:20) for 15 and 30 days, respectively; Group IV to VI mice were treated as in Group II and then fed with 50, 150, and 250 mg/kg of TG seed extract, once daily for 15 and 30 days. The mice treated with 100 ppm sodium arsenite for 2 months received three different doses of TG seed extract at two different fixation intervals.

Preparation of the *Trigonella foenum graecum* seed extract

The seeds were collected from the local market of Midnapore Municipality, West Bengal, India, and identified. A voucher specimen has been deposited at the Botany department (V-1239 MDC/2014) for record. Sundried grounded seeds (100 g) were extracted in 90% ethanol (the ratio of plant material to solvent was 1:10 m/v). The extraction was carried out at 50°C with constant stirring for 24 h. The extract obtained was evaporated to dryness and stored at 4°C until required. The yield of the dried seeds was 12.94% which was calculated by the following equation: Yield (g/100 g of dry plant material) = W1 × 100/W2, where W1 and W2 represent the weight of the extract after evaporation of solvent and the weight of the dry plant material, respectively.

Acute and subchronic toxicity studies

Groups of six mice each were fed with four doses of the ethanol seed extract, namely, 100 mg/kg, 200 mg/kg, 500 mg/kg, and 1 g/kg/day separately. Signs of general toxic effects such as weight loss, death, and abnormal behavior, if any, were observed up to 96 h after feeding for acute toxicity study. Further, for subacute toxicity studies, TG seed extract at doses of 1.2, 1.5, and 2.0 g/kg was orally administered to separate groups of six mice each once daily for 2 months. Toxic manifestations such as weight loss, seizures, death, and behavioral changes, if any, were monitored on a daily basis. At the end of 60-day period, the mice were euthanized, and macroscopic observations of heart, kidney, liver, and test were also monitored.

Preliminary phytochemical screening

The phytochemical screening for flavonoids, alkaloids, tannins, carbohydrates, reducing sugars, glycosides, and steroids was analyzed by routine procedures.[31]

Antioxidant activity (1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity) of ethanolic extract

The antioxidant activity of the TG seed extract was conducted on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity.[32] The solutions of the test extracts were prepared in ethanol. Ascorbic acid was used as standard in 50, 100, 150, 200, 400, 600, and 800 μg/ml solution. 0.002% of DPPH was prepared in ethanol, and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution (ascorbic acid) separately. These solution mixtures were incubated in dark for 45 min, and optical density was measured at 517 nm (Shimadzu UV-1800, Japan). Ethanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded, and the antioxidant activity was expressed in terms of IC_{50} (concentration of the extract/reference compound required to inhibit DPPH radical formation by 50%). All the values are the mean of three independent measurements.

Blood sampling and hematological and biochemical investigations

Blood was collected from retro-orbital plexus of mice, and serum was obtained from blood without ethylenediaminetetraacetic acid (EDTA) by centrifugation for determination of creatinine, bilirubin, gamma glutamyl transferase (GGT), and lactate dehydrogenase (LDH) activity following the method of Bonsnes and Taussky, Jendrassik and Grof, Szasz, and Gay.[33-36] Blood with EDTA samples was used for the determination of G-6PD activity as described by Lowe *et al.*[37] Liver tissue of sacrificed animal was quickly isolated and separately processed. Briefly 50 mg of the liver tissue was homogenized in 10 mL of phosphate buffer and centrifuged at 7000 *g* for 15 min in cooling centrifuge (C-24BL, REMI, Instruments, India) for the analysis of lipid peroxidation (LPO) as described by Buege and Aust,[38] aspartate and alanine aminotransferase (AST and ALT) by the method of Bergmeyer and Brent,[39,40] acid and alkaline phosphatase (ACP and ALP) by the method of Walter and Schutt,[41] catalase (CAT) according to the method of Sinha,[42] and superoxide dismutase (SOD) following the method of Kakkar *et al.*[43] Before carrying out the enzymatic estimations, the quantitative estimation of total protein was conducted by Lowry *et al.*'s procedure.[44]

Determination of hematological variables

Hemoglobin (Hb) content (mg/dL) was determined with the help of an hemometer (Marienfield, Lauda-^Konigshofen^^ Germany). For blood glucose estimation, standard glucose test kit was procured from Span Diagnostics Limited, Gujarat, India, which followed the method of Kaplan.[45] Blood urea and nitrogen (BUN) was measured by the method of Wybenga *et al*. with the kit supplied by Reckon Diagnostics Pvt. Limited, Vadodara, Gujarat, India).[46]

Determination of pro-inflammatory cytokine tumor necrosis factor-alpha and interleukin-6

The serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were determined by using the ELISA kit (R and D Systems, Minneapolis, USA) according to the procedure recommended by the manufacturer.

Procedure for histology

For the preparation of histological slides, the routine technique of paraffin sectioning of Feldman and Wolfe was followed (kidney being the major target organ of sodium arsenite) with hematoxylin and eosin staining at day 15 and day 30 only.[47]

Determination of arsenic from various tissues

As contents in various tissues (liver and kidney), urine, and blood were determined by a Perkin Elmer Analyst (AA200) Cleveland, USA) atomic absorption spectrophotometer adopting the standard AAS protocol. Each of the urine and blood samples (100 µL) and 1 mL of tissue homogenate were taken separately into 25-mL volumetric flask, to which 5.0 mL of a mixture HNO_3:$HClO_4$:H_2SO_4 (3:1:1) was added and kept for predigestion for about 2 h. Subsequently, the flasks were heated at 300°C on a sand bath. The digestion continued until a colorless liquid (0.5 mL) was obtained. All samples were performed in triplicate except blood and urine samples which were performed in duplicate due to less availability of the test materials.

Gas chromatography-mass spectrometry analysis of *Trigonella foenum graecum* seeds

Gas chromatography-mass spectrometry (GC-MS) analysis of ethanol TG seeds was conducted by a gas chromatograph coupled to a mass spectrophotometer equipped with a fused capillary column, Model No. Agilent 190915-433 CA, USA (HP-5MS, 0.25 mm × 30 m × 0.25 µm). Mass spectra were obtained by EI at 69.9 eV over the scan range m/z 50- 500 amu. The carrier gas was helium (99%) with a constant flow rate of 1 mL/min. The volume of the sample injected was 5 µL in GC-grade ethanol with an average velocity of 37 cm/s. The initial temperature of the column was 50°C for 5 min and then, it was programmed to 280°C. The total GC running time was 28 min.

Statistical analysis

Statistical comparisons were made between the positive control + Alc groups to that of TG-fed group. The significance of difference between data of the different groups was calculated by Student's *t*-test. ANOVA (SPSS 10.0 software, (SPSS Inc., Chicago, IL, USA)) was used to compare multiple groups and differences within the groups, and these were also tested for multiple comparisons by Tukey's honestly significant difference test. Because the results were more striking, we mainly focused to analyze more critically the results obtained in only As + Alc-fed series and As + TG fed at a dose of 150 mg/kg (positive) and inclined to highlight these results more than that of the other doses used and included data leaving aside those of the other doses in the table to avoid complexity; otherwise, it would have made the data look more cumbersome rendering relatively greater difficulties in data comparison and analysis. During observation and analysis of data, the observers were kept "blinded" without having any idea whether the samples belonged to the treatment or control groups.

RESULTS AND DISCUSSION

Acute and subchronic toxicity studies

From the result of the acute and subchronic toxicity studies of ethanolic extract of TG seeds in mice, no mortality was observed in any test group in 96 h up to 2 months of follow-up period. On careful daily cage-side examination, no clinical sign of toxicity was encountered in acute and subchronic treatment, and there was no behavioral change in mice that were exposed to different concentrations of ethanol seed extract of TG. The weight of mice that had undergone acute and subchronic toxicity exposure also did not vary significantly as compared to those of untreated controls. Behavioral patterns such as salivation, sniffing, corner sitting, and muzzing of 2000 mg/kg treated animals were relatively differentiable when compared to that of normal controls. Further, no macroscopic/microscopic changes were noted in the liver, kidney, and spleen of mice treated with TG seed extract in relation to the control group [Table 1].

Preliminary phytochemical screening

Analysis of the ethanolic extract of TG seeds reveals the presence of flavonoids, alkaloids, tannins, carbohydrate, reducing sugars, glycosides, and steroids [Table 2]. The presence of alkaloids and flavonoids was more glaring when compared to other compounds as revealed from the color intensity denoted by +. DPPH is used to explore the scavenging activity of compounds which are obtained from natural sources. Figure 1 indicates the radical scavenging property of TG seed extract. The IC_{50} value in the present investigation was 66.78 µg/mL.

Table 1: Mortality and symptoms of mice treated with various concentrations of ethanolic extract of *Trigonella foenum graecum* in acute and subacute toxicity studies

Dose/day	Mortality	Symptoms within 1-2 h after administration of TG
Acute toxicity study (96 h)		
100 mg/kg	0/6	Nil
200 mg/kg	0/6	Nil
500 mg/kg	0/6	Nil
1000 mg/kg	0/6	Nil
Subacute toxicity study (60 days)		
1200 mg/kg	1/6	Nil
1500 mg/kg	0/6	Nil
2000 mg/kg	0/6	Salivation, corner sitting, drowsiness, and sniffing each other

TG: *Trigonella foenum graecum*

Table 2: Preliminary phytochemical screening of *Trigonella foenum graecum* seed extracts

Chemical compounds	Seed extracts of TG
Flavonoids	+++
Alkaloids	++++
Tannins	++
Carbohydrates	+++
Reducing sugars	++
Glycosides	++
Steroids	+

TG: *Trigonella foenum graecum*, +: Minute, ++: Less abundant, +++ and ++++: Much abundant

Comparison of different biomarkers of toxicity

The activity of GGT was more in only As-fed series and As + Alc-fed series at both fixation intervals when compared to normal and As + TG-fed series. TG fed at 150 mg/kg appeared to show more modulating effect when compared to other two doses at both the fixation intervals which were statistically significant ($P < 0.05$) [Figure 2]. A similar trend was also noticed when comparing the data of LDH activity. LPO was also high in both only As-fed series and As + Alc-fed series; however, As + TG at 150 mg/kg treatment offered better protection when compared to the other two doses at both fixation intervals ($P < 0.001$) [Figure 2]. A similar observation was also noted when comparing the mean activities of AST and ALT [Figure 3]. On analysis of the results of ACP and ALP, it was noted that there was an increase in the activity of both the enzymes in liver tissues in As-fed series and As + Alc-fed series when compared to normal and other treatment series at both fixation intervals. Although there was a decline in activity in As + TG-treated series (at all three concentrations), it was not statistically significant. The activity of CAT was high in normal control mice when compared to only As-fed and As + Alc-fed series. However, treatment with TG seed extract appeared to modulate the CAT activity when compared to As-fed series and As + Alc-fed series. From the investigation, it was revealed that the dose of As + TG at 150 mg/kg appeared to offer better protection as compared to the other two doses, which was statistically significant at both the fixation intervals ($P < 0.05$ to $P < 0.001$) [Figure 3]. Oral administration of sodium arsenite decreased the SOD activity in As-fed and As + Alc-fed series when compared to TG treatment series at both the fixation intervals. Further, it was noted that As + TG at 150 mg/kg offered better protection when compared to 50 and 250 mg/kg. Administration of As + TG at 150 mg/kg resulted in increased activity of SOD when compared to As + Alc-fed series at both the fixation intervals and the increase was statistically significant ($P < 0.001$) [Figure 2].

Comparison of hematological variables

There was a decrease in the mean activities of G6PD in mice fed only As and As + Alc at both the fixation intervals when compared to normal controls and mice treated with As + TG extract, which was statistically significant. However, when the activities were compared between the treated groups, it was found that treatment with 150 mg/kg offered better protection than the other two doses, i.e., 50 and 250 mg/kg, and also when compared to Alc-fed series ($P < 0.01$) [Figure 4]. The creatinine content steadily increased from 15- to 30-day treatment intervals in only As-fed and As + Alc-fed series when compared to normal and other treatment series, which was significant. Although administration of TG at all concentrations reduced the activities of serum creatinine, analysis of the data revealed that TG treatment at 150 mg/kg concentration offered the maximum protection, which was statistically significant when compared to As + Alc-treated series ($P < 0.05$ to $P < 0.01$) [Figure 5]. A similar trend was also noticed with regard to bilirubin content [Figure 5]. There was a decrease in Hb concentration in only As-fed and As + Alc-fed series at both the fixation intervals when compared to normal mice; however, there was a slight increase in Hb concentration in the mice fed with TG extract, though it was not statistically significant [Figure 4]. The levels of serum urea and BUN also showed a similar trend which declined significantly

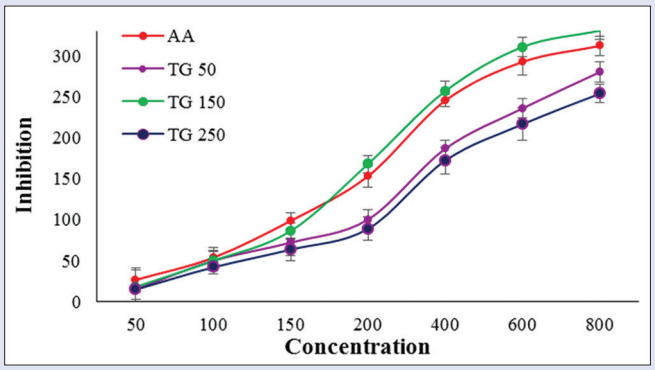

Figure 1: Percentage inhibition of alcoholic seed extract of *Trigonella foenum graecum* against ascorbic acid standard

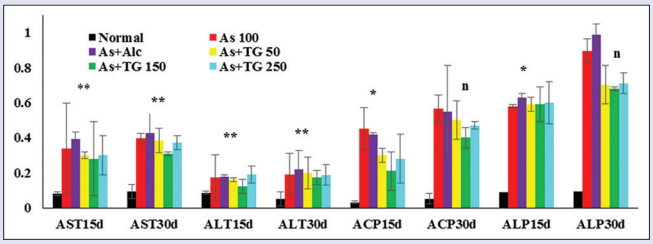

Figure 3: Mean activity of AST, ALT (mM/min/mg in different tissues), ACP, and ALP (mM phenol liberated/100 mg protein) in different treated and control series at 15- and 30-day fixation interval. AST: Aspartate transaminase; ALT: Alanine aminotransaminase; ACP: Acid phosphatase; ALP: Alkaline phosphatase. *$P<0.05$, **$P<0.01$, n=non significant

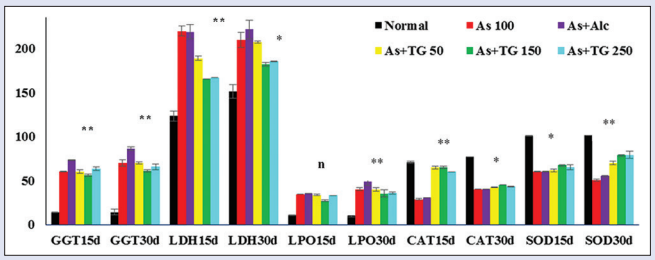

Figure 2: Mean activities of GGT and LDH (IU/L) in the serum, and LPO (nM MDA/g of tissue), CAT (nmH_2O_2 decomposed/min/mg protein), and SOD (n moles of CDNB conjugated/min/mg protein), activities in the liver of different treated and control series at 15- and 30-day fixation interval. GGT: Gamma glutamyl transferase; LDH: Lactate dehydrogenase; LPO: Lipid peroxidation; CAT: Catalase; SOD: Superoxide dismutase. *$P<0.05$, **$P<0.01$, n=non-significant

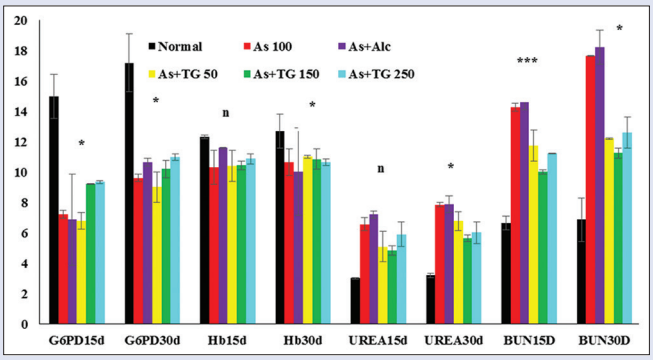

Figure 4: Mean activities of G6PD (U/g Hb), hemoglobin content (mg/dL), serum urea, and blood urea and nitrogen (mg/dL) of mice in different treated and control series at different fixation intervals. *$P<0.05$, **$P<0.001$, n=non significant

on treatment with TG seeds [Figure 4]. The levels of blood sugar were appreciably high in only As-treated and As + Alc-fed series when compared to normal controls at both the fixation intervals. However, it was reduced significantly in As + TG treated at 150 mg/kg body weight when compared to only As + Alc-fed series, vehicle of the plant extract, and also when compared to TG 50 mg/kg body weight (b. wt) and 250 mg/kg-treated series ($P < 0.05$) [Figure 6].

Comparison of pro-inflammatory cytokines

In the present investigation, the TNF-α and IL-6 production decreased in mice treated with different doses of TG at 30-day fixation interval when compared to only As-fed and As + Alc-fed mice. However, in all the groups, the production of both the cytokines was appreciably high when compared to normal controls [Figure 7]. The dose of 150 mg/kg of TG seed extract appeared to offer better protection when compared to the other two doses. Due to insufficiency of blood from As + Alc group, we could not ascertain the cytokine determination at day 15 interval which was admittedly a lacuna for reasons beyond our control.

Histological observations

The histology of normal untreated kidney at day 30 [Figure 8a] showed all typical features of the kidney; because renal tissues were primarily involved in As elimination, it was of interest to see if the normal architecture was retained in the treatment series. A decreased cellularity of the glomeruli as well as edema was found in some epithelial cells treated with only As [Figure 8b]. Some interstitial cells also showed marked edema. A similar trend was also observed in the kidneys of mice treated with As + Alc [Figure 8c]. However, edema decreased considerably in interstitial and epithelial cells, and the normal glomerular structure was found to be fairly retained in kidney sections when the mice were treated with different doses of TG extracts [Figure 8d].

Analysis of the presence of arsenic

Data on deposition of As in various tissues are provided in Figure 9. It was observed that amount of deposition of As in liver was significantly high in only As-fed and As + Alc-treated mice when compared to As + TG-treated mice. Further analysis of the urine revealed that the concentration of As removed via urine was more in As + TG-fed mice when compared to only As-fed and As + Alc-fed mice, which was statistically significant ($P < 0.01$) [Figure 9]. Urinary As concentration increases significantly after treatment with both doses of TG seed extract when compared to only As-fed and As + Alc-fed series as revealed in the present investigation ($P < 0.05$ to $P < 0.001$). When data of mice fed with different concentrations of TG extracts were compared, it was revealed that the 150 mg/kg b. wt treatment appeared to have significantly greater efficiency in the removal of As when compared to that of the other two doses.

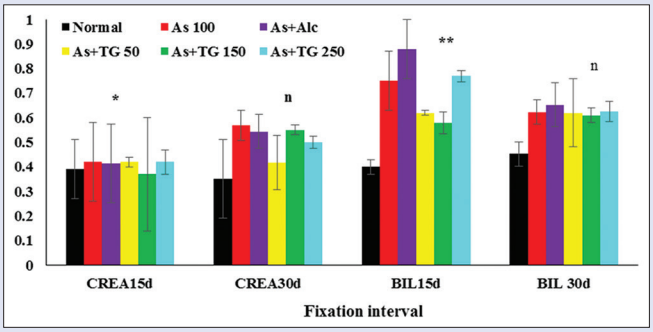

Figure 5: Mean activities of creatinine (mg/100 mL) and bilirubin (mg/dL) of mice of different treated and control series at different fixation intervals. *P<0.05, **P<0.01, n=non significant

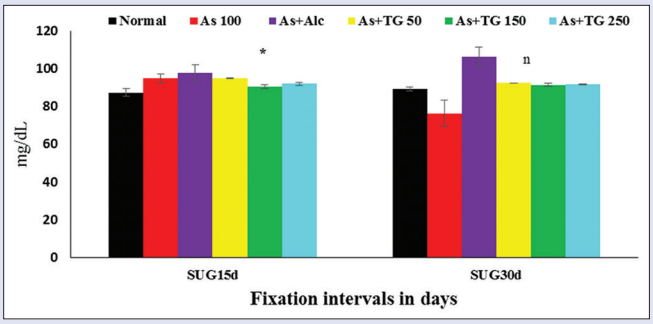

Figure 6: Mean activities of blood sugar (mg/dL) at 15 and 30 days in treated and control mice. *P<0.05, n=non significant

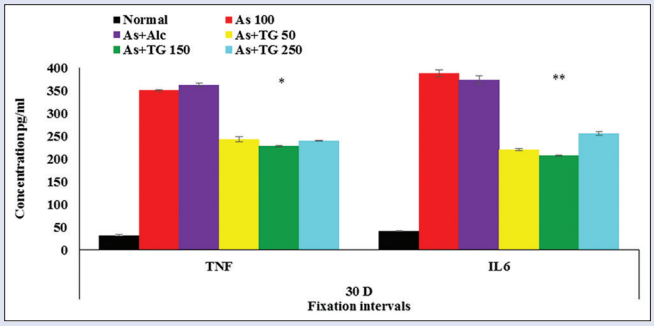

Figure 7: Effect of ethanolic seed extract of *Trigonella foenum graecum* on pro-inflammatory cytokine in arsenic-treated mice and suitable controls at 30-day fixation interval (pg/ml). *P<0.05, **P<0.01

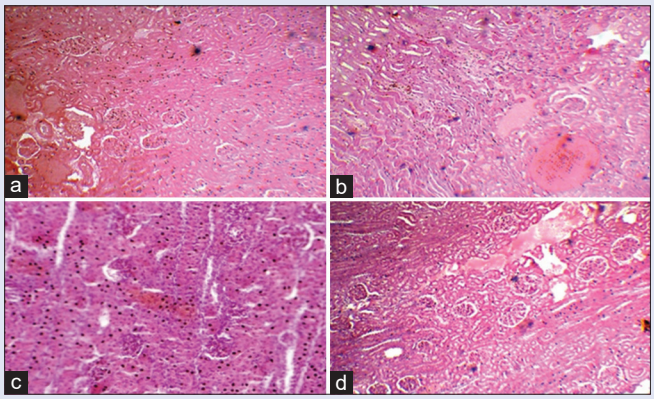

Figure 8: (a) Histological sections of untreated kidney where normal architecture was retained with intact glomerulus present (×40). (b) A decreased cellularity was observed, and edema was also evident in the epithelial cells in mice treated with only arsenic at day 30 (×40). (c) A decreased cellularity of glomeruli was observed, and marked edema was also evident in kidney of mice treated with only arsenic + alcohol at day 30 (×40). (d) Normal architecture of glomeruli was retained, and less edema was also evident in kidney of mice treated with only arsenic + *Trigonella foenum graecum* (150 mg/kg body weight) at day 30 (×40)

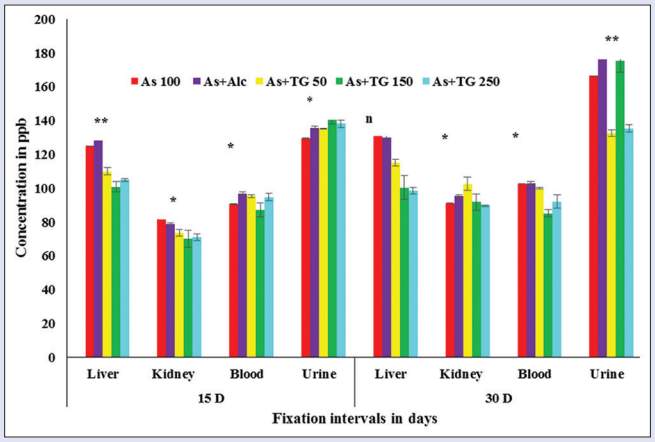

Figure 9: Mean concentrations of arsenic (ppb) in liver, kidney, blood, and urine in different series of mice at 5 and 30 day fixation intervals (n = 5). *P<0.05, **P<0.01, n= non- significant

Characterization of gas chromatography-mass spectrometry analysis

GC-MS results revealed the presence of several phytochemicals as listed in Table 3. We have found several useful compounds such as phytols and sterols such as campesterol, stigmasterol, lupeol, tetradecanal, and hexanoic acid.

DISCUSSION

In the present investigation, we have demonstrated the modulation of sodium arsenite-induced toxicity in mice by administration of various concentrations of TG seed extract by taking into consideration several biomarkers of toxicity. On analysis of the several parameters, it was revealed that there was a significant increase in several biomarkers of toxicity in only As-fed and As + Alc-treated series when compared to TG-treated series. The seeds of fenugreek have been successfully used to treat a number of ailments/diseases in the Indian system of medicine, but, unfortunately, its proven efficacy against As toxicity had not been documented earlier, which incidentally happens to be a significant finding in view of the rapidly spreading menace of groundwater As contamination. As toxicity due to chronic groundwater poisoning has started taking its huge toll by causing a variety of diseases of the dermal, cardiovascular, nervous, hepatic, hematological, endocrine, and renal systems, which is a major problem in Bangladesh, India, and many other countries.[48] As is a ubiquitous metalloid found in soil, surface water, and groundwater, but its environmental concentration increases due to natural or anthropogenic sources and once the external and internal symptoms, collectively called arsenicosis, start to manifest, there is hardly any effective medicine found in the orthodox or Western medical system to combat and reverse the effects. Further, poverty, being another major concern of the population of these regions, prevents them from taking expensive medicines or creating amenities necessary for avoiding contaminated water completely. One major cause that can be attributed to the devastating effects of chronic As feeding is its ability to generate reactive oxygen species (ROS), which in turn is involved in oxidative damage to DNA, protein, and lipids, and this ultimately leads to cell death.[49] It has been reported by several authors that oral administration of inorganic As on entering the body of animals is methylated to several metabolites, mainly dimethylarsinic acid which is eliminated from the body via urine.[50,51] The results of the present study are very encouraging because posttreatment of TG seed extracts favorably modulates As toxicity to a great extent as the TG seeds are easily available, are relatively cheap, and are thus affordable by a larger section belonging to the economically weaker echelon of the common people living in As-contaminated zone. As per results of the present investigation, the dose of 150 mg/kg TG seed extracts appears to offer better protection when compared to the other two doses.

Thiobarbituric acid reaction substances (TBARS) is a diagnostic index of LPO which is mainly due to oxidative stress and an increase in LPO at both fixation intervals in only As-fed and As + Alc-fed mice. This would clearly indicate how adversely the normal metabolic processes can be disrupted by As intoxication. TG seed extract can effectively decrease the activity of LPO, possibly owing to the presence of phenolic compounds and flavonoids in TG extract which act as scavengers of oxidizing molecules including singlet oxygen and free radicals.

To the best of our knowledge, this is the first study that demonstrates the modulating potentials of TG seed extract against As-induced toxicity in mice. In As-fed and As + Alc-fed series, there was an elevated level of AST, ALT, LPO, LDH, and GGT activities. In the present study, there is an increase in the CAT and SOD activities with a concomitant decrease in AST, ALT, LDH, LPO, and GGT activities at both fixation intervals in the TG seed extract-fed series. There are numerous scientific evidences that showed that As inhibited glucose-stimulated insulin secretions because As targets specifically β-cells, thereby inhibiting their ability to respond to glucose in blood.[52] Further, previous research showed that As decreases heme metabolism resulting in lower Hb concentration; our findings also corroborated well with the findings where Hb concentration was lowered in As-fed and As + Alc-fed mice, which was reversed in the treatment series.[53] Incidentally, till now, there are no evidence-based treatment regimens to treat chronic As poisoning although some chelators such as DMSA and diethylenetriamine-pentaacetic acid have been tested without any proven success.[54-57] Further, the chelators being toxic by themselves and also being relatively expensive can only have a restricted use. Of the different phytochemicals detected in the TG extract [Table 3], quite a few compounds are known for their reducing effects on toxicity. Presence of calyculaglycoside A in the extract might be responsible for the anti-inflammatory effect of the whole extract on the kidneys; further, isocubenol was also present in the extract which is a potent phytoconstituent that blocks the production of several pro-inflammatory mediators.[58] It has been reported earlier that calyculaglycoside A inhibits the synthesis of prostaglandin PGE2 and leukotriene, suggesting that it is a nonselective inhibitor of 5-lipoxygenase and cyclooxygenase pathways.[59] Treatment with the extract containing such components may have an anti-inflammatory effect on the kidneys. Carvacrol is a monoterpene phenolic constituent of essential oils which is also present in huge amount in the extract and is a potent anti-inflammatory substance. Carvacrol is reported to inhibit hypernociception by inhibiting migration of neutrophils and cells involved in the production of pro-inflammatory cytokine such as TNF-α and a decrease in prostaglandins.[60,61] Similar decrease in TNF-α production after treatment with the extract of TG in the present investigation might be due to the presence of carvacrol. Cytokine response to inflammation is a complex phenomenon and also depends on inflammatory stimuli.[62] Thus, presence of the abovementioned compounds in the ethanolic extract might be responsible for the inhibitory effect on TNF-α and IL-6 production.[63] Intriguingly, TG seeds offered a significant protection against As-induced elevation of blood glucose levels and also blood As levels (P < 0.05 to P < 0.001); it might be that extract of TG seeds inhibited As-mediated signal transduction pathways by inhibiting As-induced generation of ROS. Further, the phytoconstituents which are present in the extract have the ability to remove As from the binding sites, which has been reported earlier by other investigators.

Table 3: Gas chromatography-mass spectrometry data of ethanolic seed extract of *Trigonella foenum graecum* compared to Wiley 7n. 1 database and the compounds listed

PK	RT	Area Pct	Library/ID-Wiley 7n. 1 database
1	6.9986	0.1062	Benzoic acid, 2-amino-, phenylmethyl ester
2	7.2217	0.1883	Calyculaglycoside A
3	7.3133	0.2303	2-methylbutanoic acid
4	8.1029	0.1037	Pentanoic acid
5	8.2116	0.2503	1,2-Ethanediol
6	9.5506	0.0982	Isopentyl hexanoate
7	10.7293	0.101	2-Propen-1-amine, N-ethyl- Allylethylamine
8	12.1312	0.062	1-Isopropyl-2,2-dimethylpropylideneamine
9	13.4701	0.6156	Imidazole, 4-fluoro-1-methyl-5-carbhydrazino-
10	15.1467	0.0962	Tetradecane
11	15.7532	0.2061	5,5-Dimethyl-3-pyrazolidinone
12	16.1709	0.1054	N-FORMYLPROLINE METHYL ESTER
13	16.2968	0.1927	ISO AMYL BUTYRATE
14	16.5485	0.1815	Tributylamine
15	17.1493	5.5985	2,4-Dimethylpyrrole
16	18.0305	0.4996	MEGASTIGMATRIENONE 2
17	18.2651	0.2059	Propanedioic acid, diethyl ester
18	19.152	2.0299	SILACYCLOPENTANE-1,1-D2
19	19.461	4.2996	Ethyl. alpha.-d-glucopyranoside
20	19.5068	1.1743	Thiophene, Thilane, Thiolane $ Thiophane THIACYCLOPENTANE
21	19.6499	1.2799	Butanoic acid, $$ n-Butyric acid
22	19.7014	1.2545	Methyl. beta.-D-ribopyranoside $$ Ribopyranoside
23	19.7357	1.4731	Heptanoic acid (CAS) $$ Heptoic acid
24	19.8101	2.2676	beta.-D-Glucopyranoside
25	20.0447	8.3194	Ethyl. alpha.-d-glucopyranoside
26	20.491	0.8103	MOME INOSITOL
27	20.6741	0.8297	1,2-Benzenedicarboxylic acid, dibutyl ester
28	20.7599	1.1847	9,11-Octadecadiynoic acid, 8-hydroxy-, methyl ester
29	20.8457	1.7756	1,8-Dioxacyclohexane-2,10-dione, 5,6:12,13-diepoxy-8,16-dimethyl-
30	21.1318	0.4944	Hexadecanoic acid, methyl ester, Palmitic acid methyl ester
31	21.5209	0.1747	Propane, 1,2,2,3,3-pentachloro-1,1-difluoro-
32	21.6583	1.3041	n-Hexadecanoic acid
33	21.7441	0.2311	1-Nonadecene
34	21.9959	0.863	2-chloro-1,8-dihydroxy-5-methoxy-6-methyl-9H-xanthen-9-one
35	22.1618	0.1803	2-Methoxy-1-oxaspiro [4.4] nonane
36	22.6825	0.0441	Phosphonic acid, [1-[(trimethylsilyl) amino] ethyl]-, bis (trimethylsilyl) ester
37	22.7626	1.1647	9,12-Octadecadienoic acid, methyl ester
28	22.8313	1.1189	9,12,15-Octadecatrienoic acid, Ethyl Linoleolate
29	22.9343	0.256	Phytol
40	23.0201	0.203	Stearic acid methyl ester
41	23.2032	1.0975	Dodecanamide, N-(2-hydroxyethyl)- (CAS) $$ N-(2-HYDROXYETHYL)-LAURAMIDE $$
42	23.3863	9.5035	Linoleic acid ethyl ester
43	23.4607	4.9127	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)- $$ Linolenic acid, ethyl ester $$ Ethyl cis, cis, cis-9,12,15-octadecatrienoate $$ Ethyl linolenate
44	24.4163	0.2454	DODECANE, 6,6-DIDEUTERO-
45	24.5421	0.1127	D-Glucitol, 2-(acetylmethylamino)-2-deoxy-3,6-di-O-methyl-, 1,4,5-triacetate
46	24.6623	0.0483	10,11-dihydro-5H-dibenz[b, f] azepine-2-carboxaldehyde $$ 5H-Dibenz[b, f] azepine-2-carboxaldehyde, 10,11-dihydro- (CAS)
47	24.7195	2.393	3-Heptadecenal
48	24.7539	0.8071	9-(Isopropoxycarbonyl) phenanthrene
49	24.7939	2.0648	1-Benzoylamino-5-piperidinyl-1-(4-bromophenyl)-pentane
50	24.9484	1.524	Dodecanamide, N-(2-hydroxyethyl)
51	25.1716	0.2688	NORDEXTROMETHORPHAN
52	26.2816	0.1278	2-Acetyl-3-ethylpyrazine $$ Ethanone, 1-(3-ethylpyrazinyl)-
53	26.3503	1.8714	1,1',1"-Trimethyl-2,2':5',2"-tetrapyrrole $$ 2,2':5',2"-Ter-1H-pyrrole, 1,1',1"-trimethyl- (CAS)
54	26.5391	0.5583	1H-Indene, 2-butyl-5-hexyloctahydro- $$ Bicyclo[4.3.0]nonane, 8-butyl-3-hexyl- $$ 2-n-Butyl-5-n-hexyl-(hexahydroindan)
55	26.5906	0.1248	Di-n-octyl phthalate
56	27.3173	0.2255	Campesterol $$ Ergost-5-en-3-ol, (3. beta.,24R)- $$ Campesterin
57	27.6492	0.1337	6.beta. -Acetoxy-6,7-dihydro-2,3,9,10-tetramethoxy-7. alpha.-vinyl-5H-dibenzo[a, c] cycloheptene-5-one
58	27.7579	4.2918	9,12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester $$ Linolein, 1-mono-
59	27.8151	1.7494	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- $$ Linolenic acid

PK: Peak; RT: Retention time; Area Pct: Area (%)

The people living in high-risk As zones, particularly in the developing countries, are in dire need of some non-toxic remedies which can at least give them some interim relief measures till more effective measures can be discovered and implemented. Further, it is well known that diets rich in plant products are protective against various kinds of ailments; their protective ability has been attributed to the antioxidants such as

flavonoids and polyphenolic compounds. In the present investigation, we also found the presence of flavonoids and polyphenols which might have the capability to detoxify As-induced toxicity and may be due to a number of phenolic hydroxyl groups attached to the ring structures of flavonoids. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals, and lipid peroxyl radicals is important for inhibiting diseases associated with oxidative cell membrane damage and damage to proteins and DNA, thereby making it a good candidate for their health-promoting functions *in vivo*. TG seeds have numerous pharmacological properties which had also been reported by some other workers.[16] The dose recommended for use in mice in this investigation can be extrapolated in humans affected with low-grade chronic As poisoning as TG extract itself did not show any harmful effects even on chronic feeding for a long time, excluding the possibility of its own harmful effects on humans as well. Some authors also demonstrated the inability of TG extract to show any behavioral, cytotoxic, or genotoxic effects as reported by us in this study. Further, the ethanol extract of TG seeds increased the activity of SOD and CAT at both the fixation intervals. SOD quickens the dismutation of superoxide to H_2O_2 which prevents the generation of free radicals and CAT that in turn catalyzes the removal of H_2O_2 formed during SOD catalyzed reaction. The decrease in SOD and CAT activity in As-fed and As + Alc-fed series at both the fixation intervals could be due to generation of free radicals as a result of As toxicity, which in turn decreases the activity of CAT.

It has been reported by other workers also that the main constituents of the fenugreek extracts are polysaccharides, saponins, flavonoids, trigonelline, and choline. Polysaccharides act as modulators of carcinogenesis and stimulate macrophages.[64,65] To sum up, the modulations of As-induced toxicity as noted in the present investigation may be due to the combined action of phenolics, flavonoids, and polysaccharides.[66] The protective ability of co-administration of Vitamin C and Vitamin E against As toxicity, i.e., hepatotoxicity and hematoxicity, has been reported by others.[67] Recent studies also revealed that α-lipoic acid mitigates As trioxide-induced hematological abnormalities, and also concurrent administration of Vitamin A protected As-induced uterine toxicity in Swiss mice.[68,69] Patra *et al.,* 2005, reported modulation of black tea extract in As-induced toxicity.[70] On the other hand, Adetutu *et al.,* 2004, reported reduction of micronucleated erythrocytes in mice intoxicated by As when posttreated with *Hibiscus sabdariffa* extract.[71] Scientific works by other investigators revealed that *Mentha piperita* reduced the toxic effects of sodium arsenite-induced hepatopathy and also have hepatoprotective potentials.[72] Similarly, Barai *et al.,* 2007, reported that As-induced toxic effects were ameliorated by dietary supplementation of *Syzygium cumini* leaf extract.[73]

Although DMSA and DMPS are used as chelating agents for the treatment of metal toxicity, they have some limitations such as redistribution of toxic metal to other cells and organs; essential metals such as zinc, copper, and iron are lost along with toxic metals; and toxic metals are not removed from intracellular sites. Further, it has been well known that chelation therapy causes hepatotoxicity, nephrotoxicity, and increased blood pressure; therefore, alternative agents with minimal side effects are warranted.[74,75]

In the present investigation, GC-MS analysis revealed the presence of phytols, lupeol, campesterol, and stigmasterol, of which phytol is a precursor of Vitamin E which is an antioxidant. Lupeol, on the other hand, has anticancerous and anti-inflammatory properties, probably due to the positive modulation of As-induced toxicity or may be due to the combined action of these two compounds.

CONCLUSION

Thus, though the precise mechanism of the ameliorative action of TG seed extract is not yet clear, it can be safe to conclude that the combined actions of all the ingredients contribute collectively to their protective effects against As toxicity. Further works are in progress to assign the extent of specific/relative roles played by individual phytochemicals found in the TG seed extract.

Acknowledgements

The authors are grateful to Prof. Anisur Rahman Khuda-Bukhsh and Prof. Antony Gomes, both emeritus of University Grants Commission (UGC), Department of Zoology, University of Kalyani, and Department of Physiology, Calcutta University, West Bengal, and Mr. Sambhunath Maji, Assistant Professor, Department of English, Sidho-Kanho-Birsha University, for their helpful criticism and kind suggestions for improving the manuscript.

Financial support and sponsorship

This work was supported by a research grant from the UGC, New Delhi, sanctioned to SJB (No-42-566/2013 [SR]) and to DBT-BOOST, Government of West Bengal, for instrumental support.

Conflicts of interest

There are no conflicts of interest.

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