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Comparative Efficacy of *Syzygium cumini*Seed Extracts in Alleviating Arsenic-Induced Hepatotoxicity and Blood Cell Genotoxicity in Wistar Albino Rats

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***Syzygium cumini* is well known for its medicinal values in the indigenous Indian system of medicine. This study was designed to evaluate the protective effect of methanolic (SCM), ethanolic (SCE) and aqueous (SCA) extracts of *Syzygium cumini* seeds on arsenic-induced blood cell genotoxicity and hepatotoxicity in Wistar albino rats. Rats were divided into five groups: (1) control, (2) arsenic, (3) SCM, (4) SCE and (5) SCA. After completion of 60 days treatment period, comet assays were performed on isolated blood lymphocytes and serum marker assays indicative of hepatic toxicity were carried out. Arsenic exposed rats expressed significantly higherDNA damage in their lymphocytes than the unexposed rats. Increased activities of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), and decreased levels of total proteins were observed in arsenic exposed rats. Simultaneous administration of *Syzygium cumini* seed extracts significantly decreased the arsenic-induced DNA damage and hepatotoxicity. The amelioration of arsenic toxicity was more pronounced with methanolic extract compared to ethanolic and aqueous extracts of *Syzygium cumini* seeds.**

Keywords: Antioxidants, Arsenic, Genotoxicity, Reactive oxygen species, *Syzygium cumini.*

Syzygium cumini (L.) Skeels (Myrtaceae) is a tropical plant widely distributed in different countries of the world such as India, Sri Lanka, Australia and Malaysia[1].It has been attributed in the Indian folklore system of medicine to possess various medicinal properties[2].Different parts of this plant, such as fruits, seeds and leaves are reported to have antidiarrheal[3,4], hypoglycemic, antipyretic, anti-inflammatory[5,6], and antibacterial[7] properties. It has been valued in Ayurveda for possessing astringent, digestive, acrid and wound healing properties[2]. The leaves are used to strengthen gums and teeth, to treat stomachalgia, leucorrhoea, fever, strangury, dermopathy, constipation and gastropathy. The seeds and fruits are also used to treat pharyngitis, urethrorrhea, spleenopathy and ringworm infections[2,8]. Different parts of this plant are known to possess various bioactive compounds having free radical scavenging and antioxidant activities[1,51].

Arsenic is a widespread pollutant in several parts of the world. Arsenic contaminated water is reported in more than 30 countries worldwide. However, the major affected areas

are in the basins of rivers Brahmaputra, Ganga, and Meghna in India and Bangladesh and some parts of China[9,10]. In Taiwan, Argentina, Mexico, and the Indo-Bangladesh regions, drinking water concentrations of arsenic have been reported to be much above the standard (10 ìg/l) adopted by the US Environmental Protection Agency (USEPA) in 2001[11].Arsenic exists in both organic and inorganic forms in the environment. Inorganic arsenic compounds include trivalent (arsenite or As III) and pentavalent (arsenate or As V) species which are highly toxic for humans and animals, and are considered as class I carcinogens[12].Chronic human exposure to inorganic arsenicals is associated with various toxic effects including liver injury, peripheral neuropathy and increased incidences of skin, lung, liver, and bladder cancers[9,13].

As for other heavy metals, chelation therapy is the mainstream treatment for arsenic poisoning also. In chelation therapy, drugs such as British anti-Lewisite (BAL) and dimercaptosuccinic acid (DMSA) are used; however, these compounds are associated with several moderate to severe side effects including nausea, hypertension, itching, abdominal pain and changes in body temperature[14-16]. Administration of antioxidants from plant sources is reported to be highly effective in reducing arsenic toxicity[12,17,18]. Some studies reported that combined administration of antioxidants and chelating agents is also beneficial against arsenic poisoning-induced toxicity[14,19]. The recent trend is to exploit the therapeutic value of medicinal and/or dietary plants with antioxidative potential to alleviate the arsenic toxicity.

Owing to the notable antioxidative properties of *Syzygium cumini*, the present study was planned to determine the effect of various seed extracts of this plant on arsenic-induced hepatotoxicity andblood cell genotoxicity in Wistar albino rats.

MATERIALS AND METHODS

Plant Material

Seeds of *Syzygium cumini* were purchased from the local market and authenticated by Raw Materials Herbarium & Museum, NISCAIR(National Institute of Science Communication and Information Resources), New Delhi.

Seed Extract Preparation

After removing pericarps, seeds were dried at room temperature and then finely powdered. The dried seed powder was extracted with different solvents (methanol, ethanol and water) in a Soxhlet apparatus. The extractswereconcentrated in a rotary vacuum evaporator and then freeze dried. The yield of SCM, SCE and SCA were, respectively, 10.8%, 10.2% and 9.8% of the dried powdered seeds. The seed extracts were stored at -20°C until further use.

Experimental Animals

Wistar albino rats of either sex (100 - 125 g) were obtained from DFSAH (Disease Free Small Animal House), LUVAS, Hisar. Rats were kept under standard laboratory conditions with dark and light cycle (12/12 hr) and fed on a normalbalanced rat diet.The studies were approved by the Institutional Animals Ethics Committee (CPCSEA/0436) of Guru Jambheshwar University and all animal experiments were performed in accordance with the guidelines of the sameon animal experimentation. Animals were acclimatized for a week prior to the experiment.

Experimental Design

Rats were divided into five groups of 6 animals each and treated as follows:

Groups	Treatment
Control	Normal drinking water
Arsenic	Arsenic in drinking water (100 ppm) ad libitum
SCM	SCM (400 mg/kg/day) along with arsenic water (100 ppm) ad libitum
SCE	SCE (400 mg/kg/day) along with arsenic water (100 ppm) ad libitum
SCA	SCA (400 mg/kg/day) along with arsenic water (100 ppm) ad libitum

Seed extracts were administered by oral gavage. Body weight of animals, and their food and water intake were monitored throughout the treatment period of 60 days.At the end of treatment period the rats were weighed and blood was collected from the retro-orbital plexus of the eye under ether anaesthesia.

Lymphocyte Isolation

Freshly collected blood samples were diluted (1:1 ratio) with PBS (phosphate buffered saline) and carefully layered on the top of lymphocyte separation medium (LSM 1084) and centrifuged for 30 minutes at 400 x g.The buffy coat interface, which represented the lymphocytes, was aspirated and washed with PBS twice by centrifugation for 10 minutes at 250 x g. The supernatant was discarded and lymphocytes (pellet) were used immediately for the comet assay.

Comet Assay

The comet assay was performed according to Singh et al.[20],with slight modifications.150 µl of 0.5% NMA (normal melting agarose) was layered on to precleaned microscope slides and dried at 65 °C for 10 min.A second layer containing isolated lymphocytes resuspended in 75 µl of 0.5% LMA, was placed on the NMA precoated slides and solidified at 4 °C for 10 min. The slides were covered with 0.5% LMA and stored at 4 °C for 15-20 min.Afterwards the slides were placed in freshly prepared lysing solution (2.5 M NaCl , 100 mM Na_2EDTA, 10 mM Tris, 1% Triton X-100, 10% DMSO and pH 10-10.5) at 4 °C for 2 h in the dark.Following lysis, the slides were immersed in an electrophoretic buffer (300 mM NaOH , 1 mM Na_2EDTA, pH 13.5) for 25 min at 0 °C and electrophoresed in the same buffer for next 20 min (24 volts, 300 mA). Electrophoresis was conductedunder dim light to prevent additional DNA damage. After that, slides were rinsed with 0.4 M Tris (pH 7.5) twice for 5 min, fixed for 3 min in absolute ethanol and stained with 0.4 µg/ml ethidium bromide.Comet images were observed at 400× magniûcation with a fluorescence microscope (Olympus CX 41). For each sample, images of randomly selected 50 cells were examined. Open Comet software was used for DNA damage quantification by analysis of the tail percent DNA, tail moments and tail lengths.

Activities of Serum Markers

Commercially available diagnostic kits were used for assaying the activities of serum ALT, ALP, AST and total proteins.

Statistical Analysis

Dataanalysis was performed using One-way ANOVA followed bypost-hoc Tukey's test. The differenceswere considered statistically significant at $P<0.05$.

RESULTS

The effects of arsenic on body weight gain in control and experimental rats are depicted in Fig. 1. In arsenic-only treated rats, body weight gain percent was lower than control, SCM, SCE and SCA groups. Among experimental groups, body weight gain percent was highest in SCM group.

Activities of serum ALP, AST and ALT were significantly higher in arsenic-only group than in controls, while the same activities were all

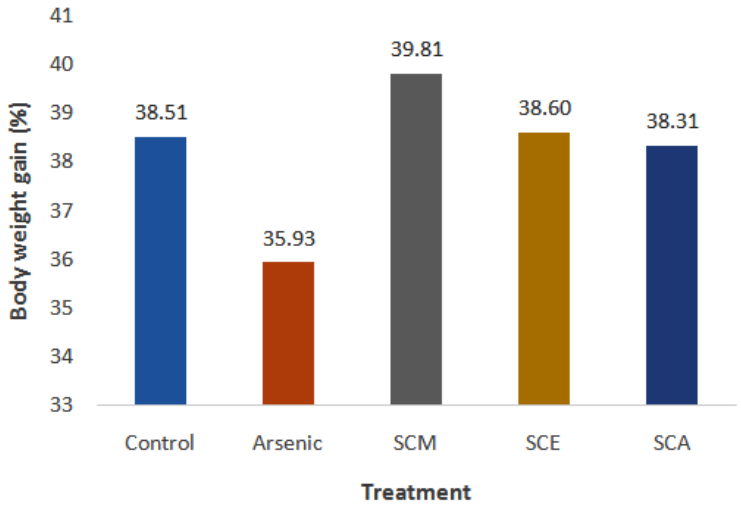

Fig. 1. Changes in body weight gain in different experimental groups; Values are expressed asmeanswith n = 6 rats per group

less in groups treated with *Syzygium cumini* seed extracts along with arsenic. Activities of ALP and AST were significantly lower in SCM, SCE and SCA groups compared to arsenic-only group. Serum total protein was significantly lower in the arsenic-only group than in control group and significantly greater in SCM, SCE and SCA groups than in arsenic-only group (Fig. 2).

DNA damage in individual bloodlymphocytes wasassessed by measuring various comet assay parameters viz.; tail percent DNA, tail moments and tail lengths. Fig. 3 depicts the value of percent tail DNA in different treatment groups. Tail percent DNA was significantly greater in arsenic group than in control. Co-administration of *S. cumini* seed extracts (SCM, SCE, and SCA) significantly reduced the tail percent DNA.A significant increase in tail moment was observed in arsenic intoxicated rats as compared to control rats. However, tail

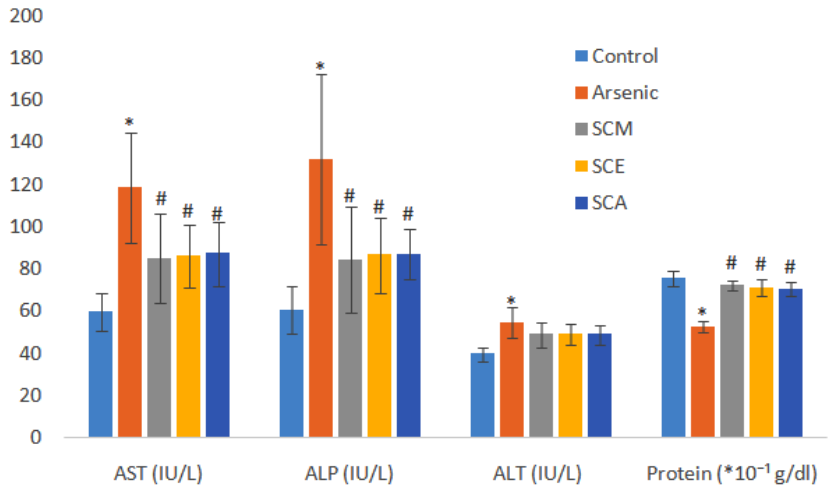

Fig. 2. Serum biomarkers activity in different treatment groups. # p<0.05 compared with the arsenic group, * p<0.05 compared with controls; Data are expressed as means ± SDs, n = 6 rats per group

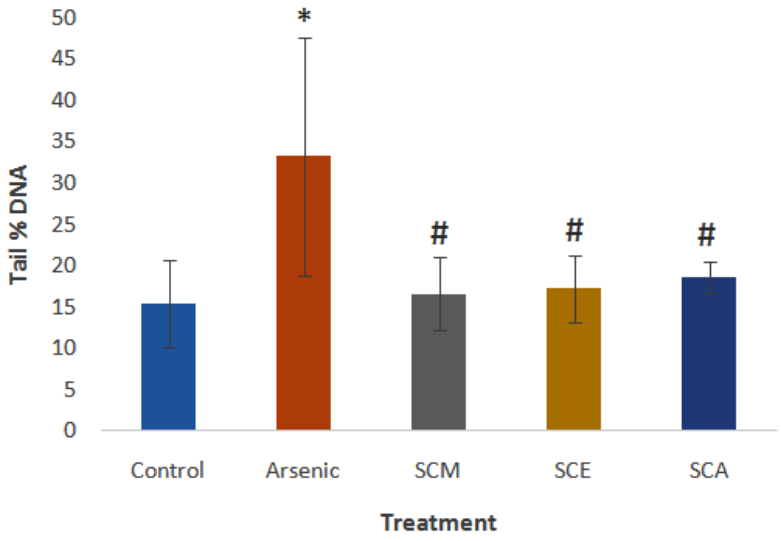

Fig. 3. Values of tail percent DNA in different treatment groups; # p<0.05 compared with the arsenic group, * p<0.05 compared with controls; Data are expressed as means ± SDs, n = 6 rats per group

moment values were significantly reduced in SCM, SCE and SCA group rats (Fig. 4). Similarly, the mean comet tail length was significantly higher in the arsenic-treated rats than in untreated rats, while the same values were significantly lower in SCM, SCE, and SCA rats than in arsenic-only exposed rats (Fig. 5). Among experimental groups treated with both arsenic and *S. cumini* seed extract, the mean values of tail percent DNA, tail moments and tail lengths were lowest in SCM, followed by SCE and SCA groups.Fig. 6 illustrates the representative comet assay images obtained by fluorescent microscopy.

DISCUSSION

The present study reveals substantial amelioration of arsenic-induced blood cell

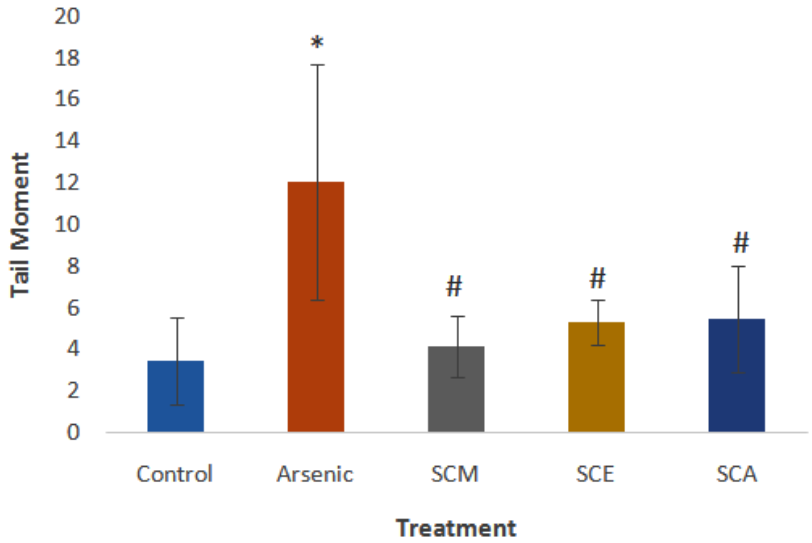

Fig. 4. Tail moment values in different treatment groups; # p<0.05 compared with the arsenic group, * p<0.05 compared with controls; Data are expressed as means ± SDs, n = 6 rats per group

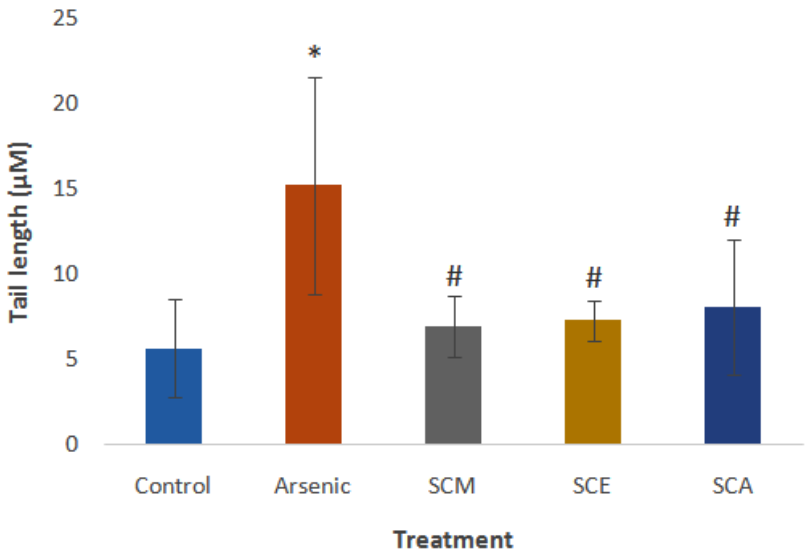

Fig. 5. Tail length values in different treatment groups; # p<0.05 compared with the arsenic group, * p<0.05 compared with controls; Data are expressed as means ± SDs, n = 6 rats per group

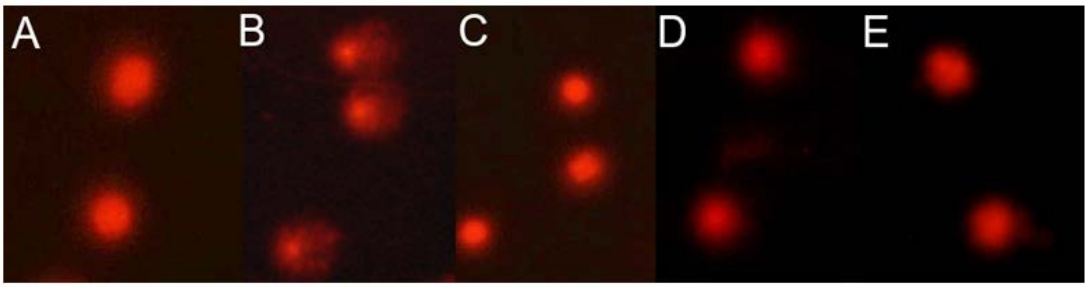

Fig. 6. Representative comet assay images obtained by fluorescent microscopy; **Groups:** (A) Control (B) Arsenic (C) SCM (D) SCE (E) SCA

genotoxicity and hepatotoxicity by various seed extracts of *Syzygium cumini*. Our results are corroborated by previous studiesthat have reported protective activity of antioxidant-rich plants such as *Emblica officinalis*, *Camellia sinensis* and *Trichosanthes dioica*against arsenic toxicity[21-23].The present findings also support the modulatory effects of *Syzygium cumini* on genomic damage and oxidative stress induced by various agents such as radiations[24], DMBA(7,12-dimethyl benz(*a*)anthracene) and urethane[25], among few others.

Arsenic is an established carcinogen present ubiquitouslyin the environment. In addition to its carcinogenic effects, long-term arsenic exposure is associated with hyperpigmentation, hyperkeratosis, type II diabetes mellitus, neurological damage, and black foot and cardiovascular diseases[26-30]. Chronic arsenic exposure leads to accumulation of arsenic in various body organs, primarily the kidneys, liver, lungs and skin, which adversely affect these organs[31-35].Reduction in body weight is alsoobserved in arsenic exposed animals which is supposed to be caused by oxidative damage to body cells and tissues[23,36]. Our results, too, revealed a decline in the bodyweight of arsenic challenged rats compared with controls. However, the combined treatment of arsenic and *Syzygium cumini* seed extracts resulted in body weight recovery towards control levels. Body weight gain was maximum in SCM, followed by SCE and SCA groups which indicates that methanolic seed extract was most effective in maintaining general body weight and thereby reducing arsenic-induced toxicity in rats with maximum potential among all extracts.

Liver is a potential target organ of arsenic toxicity[13,22]. Arsenic-mediated increase in activities of serum ALT, AST and ALP indicates hepatic toxicity and these results agree with previous findings[6,22]. Administration of *Syzygium cumini* seed extracts in arsenic exposed rats significantly restored the activities of these biochemical variables. Serum total protein levels were significantly less in arsenic exposed rats than the controls and this difference might be due to damaging effect of arsenic on hepatic cells or alterations in protein synthesis and/or metabolism[6]. Treatment with *Syzygium cumini* seed extracts significantly increased serum total protein levels towards normal in arsenic treated rats. Methanolic seed extract was most effective in decreasing arsenic-induced hepatic toxicity followed by ethanolic and aqueous extracts.

The comet assay is a simple, fast and reliable method for detection of DNA strand breaks in individual cells[20,37].The present results from comet assay show that arsenic exposed rats exhibited significantlymphocyte DNA damage when compared to the controls. Our results are in line with earlier reports demonstrating considerable DNA damage in arsenic intoxicated animals and humans. Balakumar et al.observed increased DNA strand breaks in liver, blood, bone marrow and kidney cells of rats challenged by sodium arsenite[38]. Elevated levels of DNA damage were detected by DNA fragmentation assay in hepatic cells of experimental rats subchronicaly exposed to arsenic[39].In another study, significant DNA damage in peripheral blood lymphocyteswas reported in a population exposed to chronic arsenic through

contaminated drinking water in West Bengal, India[40]. The increased lymphocytic DNA damage in arsenic intoxicated rats, as observed in our experiment, was markedly decreased in rats treated with *Syzygium cumini* seed extracts along with arsenic.Methanolic seed extract provided the maximum protection followed by ethanolic and aqueous seed extracts against the arsenic-induced DNA damage.

The exact mechanism of arsenic toxicity is not yet fully understood; however, the evidences suggest that oxidative stress is involved in arsenic-induced DNA damage and toxicity of various organs.Arsenic can disturb the natural oxidation/ reduction balance through several mechanisms involved in intricate redox reactions with endogenous oxidants and antioxidant systems of cells[41].Arsenic metabolism generates free radicals and reactive oxygen species (ROS) which induce cell signaling and transcription factor activation eventually leading to gene mutations, DNA strand breakage, sister chromatid exchange, generation of micronuclei and chromosomal aberrations[12,42]. It is suggested that superoxide anion($O_2^{\bullet}${)is the primary ROS induced by arsenic in various cellular systems; which triggers formation of other ROS such as hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$). H_2O_2 is converted to highly reactive $^{\bullet}OH$ radicals via Fenton reaction.$^{\bullet}OH$ radicals formed in vicinity of DNA may react with deoxyribose backbone of DNA or with DNA bases causing DNA strand breaks or producing damaged bases[42,43]. Enhanced nitric oxide (NO) production induced by arsenic also plays an important role in oxidative damage to DNA[44,45]. 8-hydroxy-22 -deoxyguanosine (8-OHdG) is a sensitive biomarker of ROS induced oxidative DNA damage and its elevated levels have been reported in various biological systems exposed to arsenic[42,46].Arsenic inhibits DNA repair processes which potentiate thegenotoxicity of other DNA damaging agents such as UV radiation, X-rays and benzo[a]pyrene[47-49].Arsenic-induced oxidative stress may lead to altered DNA methylation and genomic instability resulting in a higher risk of carcinogenesis[36,50].

The protective action of *Syzygium cumini* on arsenic-induced blood cell genotoxicity and hepatotoxicity may be attributed to the presence of various active phytochemicals such as triterpenoids, kaempferol, ellagic acid, myricetin,quercetin and acetyl oleanolic acid in the seeds of this plant[1,6].Most of these compounds are reported to exhibit free radical scavenging and antioxidant properties[51-54], which might have protected the animals against arsenic toxicity, probably by augmenting endogenous antioxidants[12,55], and/or by altering apoptotic pathways[12], and/ or by directly scavenging DNA-damaging free radicals.The polyphenol ellagic acid is reported to possess antioxidant, antimutagenicand chemopreventive activities[52,56]. In a previous study, ellagic acid potentially inhibited the lipid peroxidation induced by radiation in the liver of mice[57].The ûavonoids kaempferol, quercetin and myricetin are potent antioxidants which protect cells by scavenging $^{\bullet}OH$ radicals, nitric oxide and superoxide anion, and by inhibiting lipid peroxidation[53,56,58-61]. They possess reactive hydroxyl groups and stabilize various ROS by donating hydrogen atom[53]. Free radical scavenging by flavonoids decreases production of highly damaging peroxynitrite by preventing reaction of nitric oxide with free radicals[54].Myricetin has been reported to haveeven higher antioxidant capacity than Vitamin E (D-á-tocopherol)[62]. Our results are in consonance with previous reports indicating ameliorative effects of antioxidantssuch as tetrahydrocurcumin, resveratrol, and vitamins C and E on arsenic-induced toxicityeither *in vivo*[36,38] or *in vitro*[63].We alsohave reviewed the therapeutic potential of various plant-based antioxidants in arsenic genotoxicity, which further supports the results of this study[12].

CONCLUSIONS

From the observations, we conclude that methanol, ethanol and aqueous seed extracts of *Syzygium cumini* mitigated arsenic-induced blood cell genotoxicity and hepatotoxicity in Wistar albino rats. Among all, methanol extract was the most effective in alleviating arsenic toxicity. The findings here support the growing evidence that antioxidant-rich plant sources exhibit protective effects against oxidative damage to DNA and other cellular components.

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