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Research Article

In-Vivo Anticlastogenic and Hepatoprotective Property of Amla (*PhylanthusEmblica* / *EmblicaOfficinalis*) Against Arsenic Induced Toxicity in Mice

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Abstract

Phylanthusemblica or *Emblicaofficinalis*, commonly known as Indian gooseberry or Amla, belongs to the family phyllanthaceae, is known as richest source of vitamin C. The medicinal properties of Amla were well known since ancient period and were reported in ancient Indian Ayurvedic Medicine for preventing infection, hair treatment and different skin disease, Since early 1980s, an alarming problem of ground water arsenic (As) contamination has devastated eastern Indian regions. Arsenic is a known human carcinogen and an environmental pollutant known to cause adverse health effects such as liver injury, neurotoxicity, increase risk of cancers of skin, lung, bladder and liver. As many natural herbs, which can be consumed through our diet, possess excellent chemo-preventive properties, our present study was conducted to examine the anti-clastogenic and hepato-protective properties of Amla extract against Arsenic induced chromosomal aberration and hepato-toxicity in mice *in vivo*. To check*in vitro* total antioxidant property of Amlaextract, phosphomolybdenum method was performed. Our result demonstrated that, orally administered Amla extract (60mg/kg/b.w.) are effective in counteracting the clastogenecity of the most potent form of arsenic, sodium arsenite in mice *in vivo*. It also shows good amount of hepato-protective property against sodium arsenite. These results suggest that the use of Amla, which have a good antioxidant property, in diet, may give an effective protection against the health crisis generated by arsenic.

Keywords: *Emblicaofficinalis,* Arsenic toxicity, Anti-clastogenecity, Hepato-protective, Antioxidant property.

INTRODUCTION

Arsenic is widely distributed in nature and known as the most abundant pollutant with complex metabolism. It principally occurs in both organic and inorganic forms. Organic arsenicals are the least toxic ones whereas an inorganic arsenical compound arsenite, considered to be the most toxic form in comparison to less toxic arsenate. The spectrum of arsenic compounds helps its wide occurrence in the environment, results the human exposure to the metalloids throughout the world. Different investigations revealed the most common route of entry for inorganic arsenicals in human is through drinking water[1].

Arsenic is classified as Group I carcinogen in humans as it causes a wide range of toxic effects by chronic exposure[2]. Chronic arsenic toxicity through arsenic contaminated ground water has been determined the most devastating problem in the current century throughout the world especially in Bangladesh and several districts of west Bengal, India[3]. For this purpose, a new line of research has been developed to identify different method for intercepting or preventing the harmful effect of such exposures.

In vitro studies showed that arsenic induced sister chromatid exchanges and chromosomal breakage in mammalian cells[4,5]. The exposure of arsenic can causes non-random chromosome aberrations, changes in ploidy of cells, induced cancer by associated DNA damage and arsenite intoxication can compromise the integrity of hepatocytes in different mammalian experimental animals[6-9].

There is nopropermedicationavailable for chronic arsenic toxicity. Prevention of arsenic contamination in the ground water along with reduction of the consumption of contaminated drinking water is the preliminary way to fight against arsenic toxicity. Investigations show that Safe drinking water, nutritious food and physical exercise

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can also improve the situation[10]. Recent studies have been explored the dietary inhibitors of mutagenesis and/or carcinogenesis for the prevention of human cancer. For this purpose, different medicinal plant product enriched with active phytochemicals and antioxidants can be used as dietary supplements for the management of arsenicosis and several dietary intervention programmes have been initiated in endemic areas of the World to minimize the health crisis generated by arsenic[11,3].

Amla or Amlaki (*Emblicaofficinalis*) is commonly known as Indian gooseberry, belongs to the family *Euphorbiaceae*, native to India preferably grown in the dry region of the tropical and subtropical regions including Sri Lanka, China, Malaysia, Southeast Asia, Pakistan and Uzbekisthanand other areas of Middle East. It is a medium sized plant with maximum of eighteen meter height. The branches are scattered around the slightly curved trunk. The leaves are lighter in weight, linear and long in shape and smell like lemon. It has a yellowish green or pinkish flower and the fruit is pale yellow in colour, round in shape and has six vertical stripes. According to Indian mythology it is the first tree to be created in the universe[12-14].

The fruits of Emblica*officinalis* is widely used in Ayurveda since the ancient periods and known as wonder fruits to boost up body defence mechanism. It also used in traditional Unani, Siddhya and alternative folk medicine for its therapeutically important natural products. The wide spectrum of its beneficiary effect also reported in cases of anaemia, ulcer, heart trouble, liver treatment, diabetes and cancer. Presence of several active phytochemicals like alkaloids, tannin, flavonoids, polyphenols, vitamins and many more compounds make these fruits as applicable as chemo-preventive, gastro-protective, cytoprotective, immune-modulator, analgesic and anti-oxidation agent. Additional related benefits are the ability to lowering down the blood cholesterol level, improving ophthalmic disorder, and enhancing memory and helpful in neutralizing snake venom. The wide beneficial role of *Emblicaofficinalis*fruits as well as the richest source of Vitamin C made it as an ideal candidate as a herbal medicine[15-17].

As the study of this wonder fruit is still continuing, the present investigation was taken to perform a combined study of anti-clastogenic, hepatoprotective and antioxidant potentials of *Emblicaofficinalis*fruit extract and its effect on sodium arsenite-induced clastogenicity and hepatic-toxicity in order to enrich the existing dietary intervention attempts in as endemic regions.

MATERIALS AND METHOD

Selection and maintenance of experimental model

Eight week-old healthy, laboratory-bred, Swiss Albino mice (*Musmusculus*), both males and females of body weight ranging 25±2gram(g) were maintained at temperature 28±2°C (Degree Celcius) under 12 hours light /12 hours dark cycle with access to bengal gram whole and powdered, bread and tap water *ad libitum*. The experiments were followed by the guideline of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals), WBUHS (West Bengal University of Health Science) and approved by Institutional Ethical Committee.

Test Chemical

Sodium arsenite (CDH Labortory Reagent) was dissolved in distilled water and administered intraperitoneally.

Preparation of Extract

Amla was extracted in the laboratory by the following method. Fresh Amla was collected from the local market; washed thoroughly in running tap water; then sliced into smaller pieces and smashed along with of distilled water (20millilitrel/100g). The solution was filtered using Whattman 0.1 filter paper and the crude amla extract was collected. The extract was then stored at 4°C for further use.

Experimental Design for Anticlastogenicity Test

Five different treatment groups were made. Six healthy mice (three males and three females) were randomly selected for each treatment group. The dosage details of each group are tabulated in Table1. Amla extract was administered in gavage while MMC (Mitomycin C) and $NaAsO_2$ (Sodium Arsenite) were administered by intraperitoneal route. After 24 hours of the last dose the mice were sacrificed, the bone-marrow cells were collected and chromosome preparation was done from it followed by conventional Giemsa staining. The chromosomes were observed under the microscope and results were documented[18].

Table 1: Dosage details for Anti-clastogenicity Test

	Group 1 MMC (+ ve control)	**Group 2** $NaAsO_2$	**Group 3** CAE	$NaAsO_2$	**Group 4** CAE	**Group 5** Vehicle (- ve control)
Day 1			+		+	

Day 2			+		+
Day 3			+		+
Day 4			+		+
Day 5			+		+
Day 6			+		+
Day 7	+	+	+	+	+

MMC: Mitomycin C 1milligramg(mg)/kilogramg(kg)/body weight (b.w); $NaAsO_2$: Sodium arsenite (3.8mg/kg/b.w.);CAE: Crude Amla Extract (60mg/kg/b.w.); +: Dose given

Experimental Design for Hepatoprotective Test

Five different treatment groups were made. Six healthy mice (three males and three females) were randomly selected for each treatment group. The dosage details of each group are tabulated in Table 2. Amla extract was administered in gavage while CCl_4(Carbon Tetrachloride) and $NaAsO_2$ were administered by intraperitoneal route. After 24 hours of the last dose the treated mice were sacrificed, and whole blood was collected by heart puncture, followed by serum separation. Various biochemical tests were performed accordingly by Kit Method[19].

Table 2: Dosage details for Hepato-protective Test

DAYS	**Group 1 CCl_4 (+ ve control)**	**Group 2 $NaAsO_2$**	**Group 3 CAE**	**$NaAsO_2$**	**Group 4 CAE**	**Group 5 Vehicle (- ve control)**
Day 1			+		+	
Day 2			+		+	
Day 3			+		+	
Day 4			+		+	
Day 5			+		+	
Day 6			+		+	
Day 7	+	+	+	+	+	

CCl_4: Carbon tetrachloride (1.5ml/kg/b.w.); $NaAsO_2$: Sodium arsenite (3.8mg/kg/b.w.);CAE: Crude Amla Extract (60mg/kg/b.w.); +: Dose given

Experimental Design for Total Antioxidant Study

The total antioxidant activity of crude amla extract was evaluated according to Prieto*et al*., 1999. 0.3 ml of crude extract (10, 20, 30, 40, 50microlitre (µl)) were mixed with 3 ml assay mixture which contain 4 mill mole(mmol)/Litre (L) ammonium molybdate, 0.6 Mole (mol)/L sulphuric acid and 28 mmol/L sodium phosphate. The mixture along with test samples was incubated at 95°C for 90 minutes in water-bath. After cooling to 25°C, absorbance of the final solution was measured at 695 nm (nanometer) wavelengths in spectrophotometer (Beckman). Vehicle (distilled water) was used as blank and ascorbic acid as positive control[20].

Statistical Analysis

The results were expressed as Mean ± SE (Standard Error) and statistical analysis was carried out by One-way ANOVA (Analysis of Variance), P (Level of Significance)< 0.05 was considered significant. To confirm the differences occurred between experimental groups for in vivo clastogenecity study, Tukey's HSD (Honest Significant Differences) test was performed.

RESULTS

Result of Chromosomal Aberration Test

The chromosomal aberration test indicated that amla extract was capable of reducing the chromosomal aberration induced by sodium arsenite, and showed the anticlastogenic property against arsenic induced clastogenecity. When tested separately,it did not show any increase in the frequency of chromosomal aberration in human peripheral blood culture. MMC used as a positive control, showed high number of aberrant chromosomes in human peripheral leukocytes in comparison to sodium arsenite, whereas crude amla extract prevented chromosomal damage to some extent as revealed from decreased frequency in chromosomal aberration. The result of chromosomal aberration test was summarized in Table-3. The different kind of damage and aberrations were depicted in Figure: 1. The graphical representation shows the Effect of Amla extract on Chromosomal Aberration Induced by sodium arsenite in Figure: 2

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Table 3: Shows the observed chromosomal aberration in different experimental group

SL No	Test Groups	Observed Chromosomal Aberrations
1	(Group-1)Vehicle (-)ve control	0.11 ± 0.02
2	(Group-2)Crude Amla Extract	0.09 ± 0.04
3	(Group-3)Amla extract + $NaAsO_2$	0.21 ± 0.03
4	(Group-4)Only $NaAsO_2$	0.49 ± 0.05
5	(Group-5)MMC (+)ve control	0.53 ± 0.03

± = Mean SD, N=3 ; *P<0.05 significant, $NaAsO_2$-Sodium Arsenite, MMC- Mitomycin C, (+)ve= Positive, (-)ve= Negative

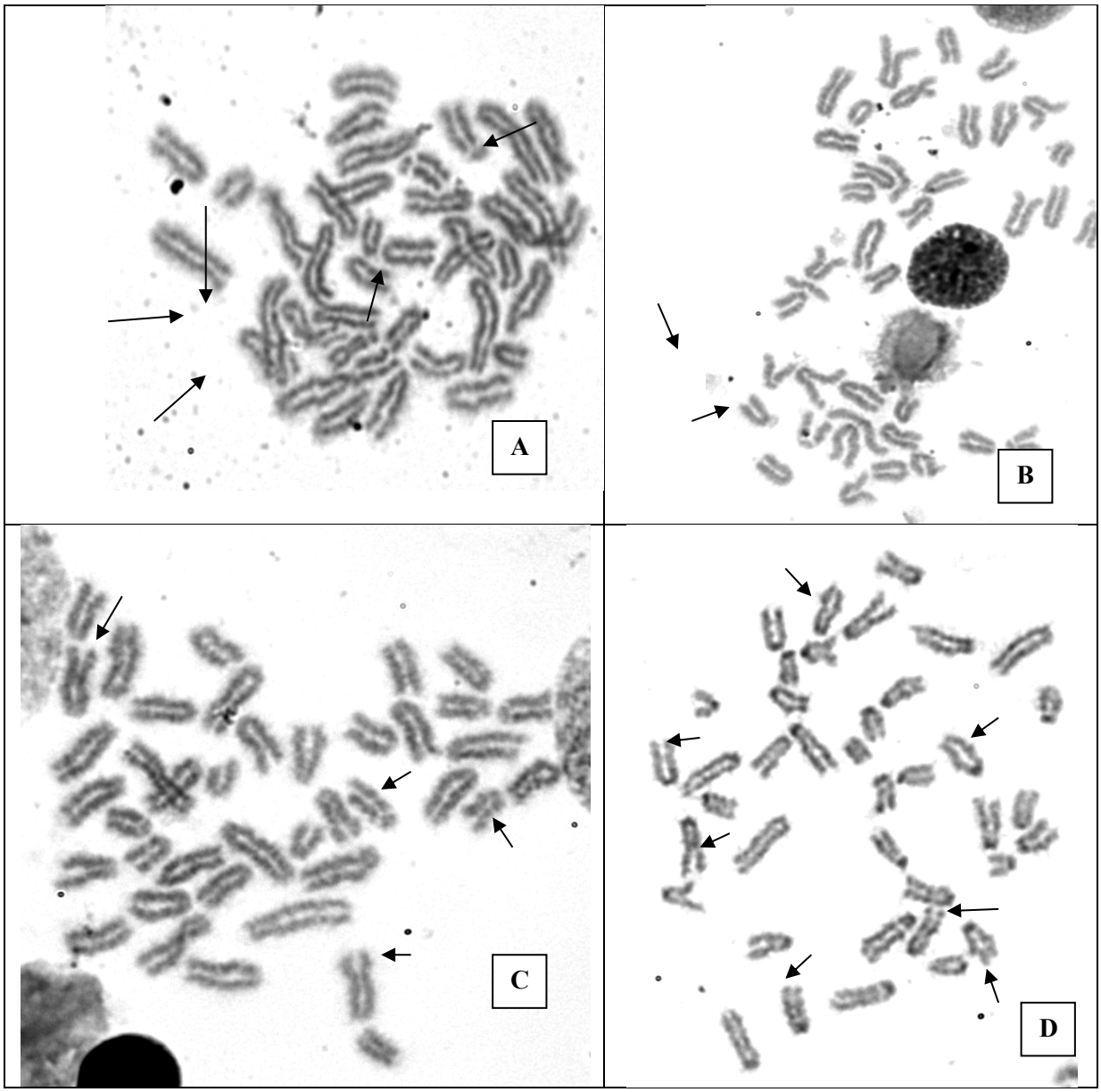

Figure 1: Microphotographs showing metaphase chromosomes of mice depicting different kinds of damages and aberrations A- Chromosome Gap, Chromatid Break, Tetrad Formation, Fragmentation. B- Chromatid Gap, Chromatid Break. C- Chromosome Gap, Chromatid Break. D- Chromosome Gap, Chromatid Break, Fragmentation, Ring Formation

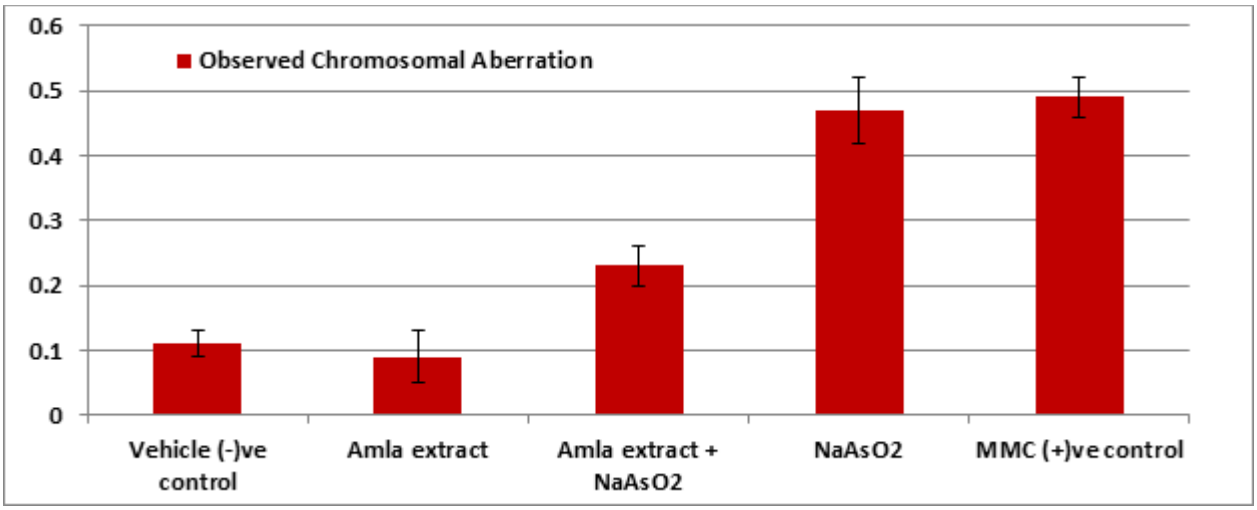

Figure 2: Graphical representations show the observed chromosomal aberration in different experimental groups

Result of Hepato-Protective Test

The hepatoprotective test indicated that amla extract was capable of reducing the hepatotoxicity induced by sodium arsenite, or in other way it showed the hepatoprotective property against arsenic induced hepatotoxicity. When tested separately, it did not show any increase in the frequency of liver function parameters. Carbon tetra chloride used as a positive control, showed high hepatotoxic property in comparison to sodium arsenite, whereas crude amla extract prevented liver damage to some extent as revealed from decreased level of liver function parameters. The result of haepaprotective test was summarized in Table-4. The graphical representation shows the Effect of Amla extract on SGOT (Serum Glutamic-Oxaloacetic Transaminase), SGPT (Serum Glutamic-Pyruvic Transaminase) in. Figure: 3whereasthe Effect of Amla extract on Total Billirubin and Direct Billirubin in Figure: 4.

Table 4: Shows the observed values of SGOT, SGPT, Total Billirubin and Direct Billirubin in different experimental group.

Sl No	Test Groups	SGOT (U/L)	SGPT (U/L)	Total Billirubin (mg/dl)	Direct Billirubin (mg/dl)
	Vehicle (-)ve control	75.40 ± 0.26	33.50 ± 0.06	0.14 ± 0.05	1.72 ± 0.07
	Amla extract	57.40 ± 0.14	31.00 ± 0.04	0.12 ± 0.02	1.62 ± 0.05*
	Amla extract + $NaAsO_2$	154.96 ± 0.22	76.00 ± 0.05	0.21 ± 0.08	1.76 ± 0.08*
	Only $NaAsO_2$	210.33 ± 0.16	103.66 ± 0.11	0.33 ± 0.06	2.17 ± 0.11*
	CCl_4 (+)ve control	222.21 ± 0.11	119.14 ± 0.13	0.41 ± 0.03	2.69 ± 0.06

± = Mean Standard Deviation, N (Total Number) =3, $NaAsO_2$-Sodium Arsenite, CCL_4= Carbon tetra chloride, dl= decilitre

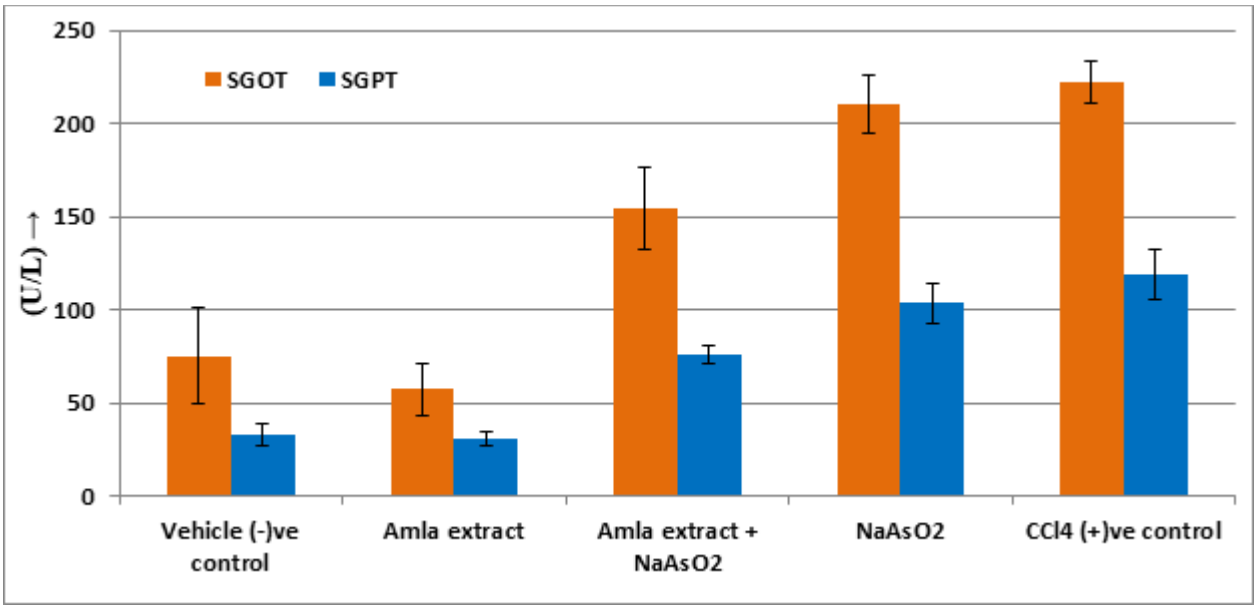

Figure 3: Graphical representation shows the observed values of SGOT, SGPT in different experimental groups

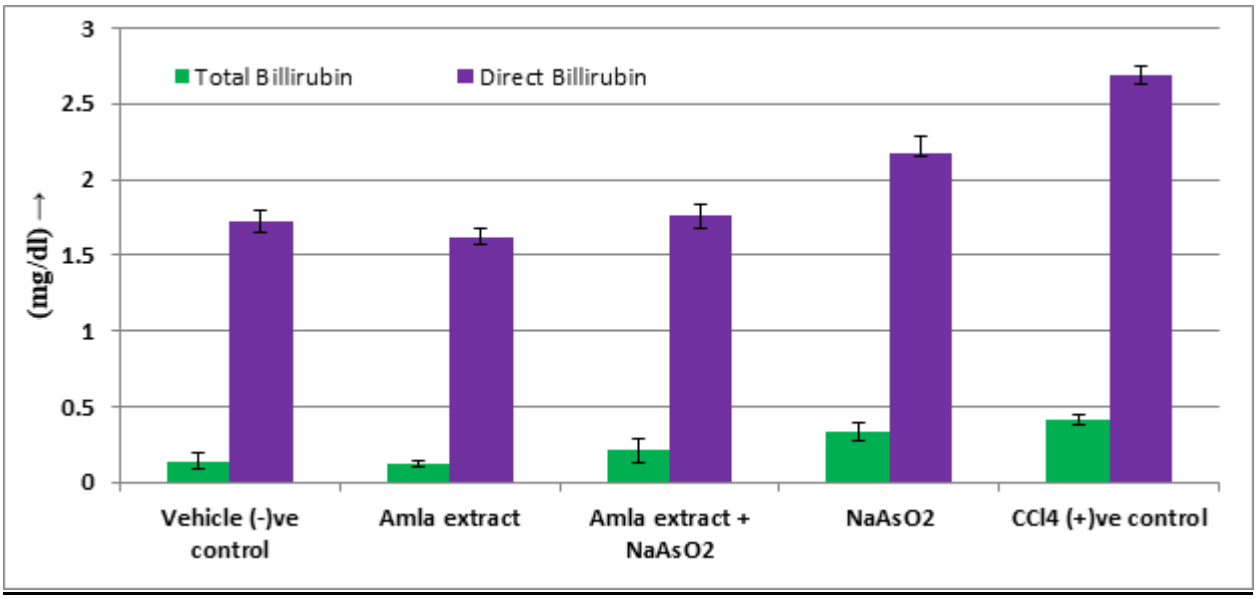

Figure 4: Graphical representation shows the observed values of Total Billirubin and Direct Billirubin in different experimental groups

Result of Total Antioxidant Activity Study

In the present study, five different concentrations of crude amla extract were taken from the stock solution for total antioxidant assay by phosphomolybdenum method. The detailed results were depicted in Table-5. The graphical representation of total antioxidant property of crude amla extract by Phosphomolybdenum Method was represented in Figure-5.

Table 5: Shows the observed Antioxidant Activity in Percentage (%) in different concentrations of Amla extract

Concentrations	Antioxidation Activity (%)
10 µl CAE	40.03 ± 0.02
20 µl CAE	53.71 ± 0.04

30 µl CAE	61.52 ± 0.04
40 µl CAE	72.26 ± 0.03
50 µl CAE	82.03 ± 0.05

± = Mean SD, N=3, CAE= Crude Amla Extract

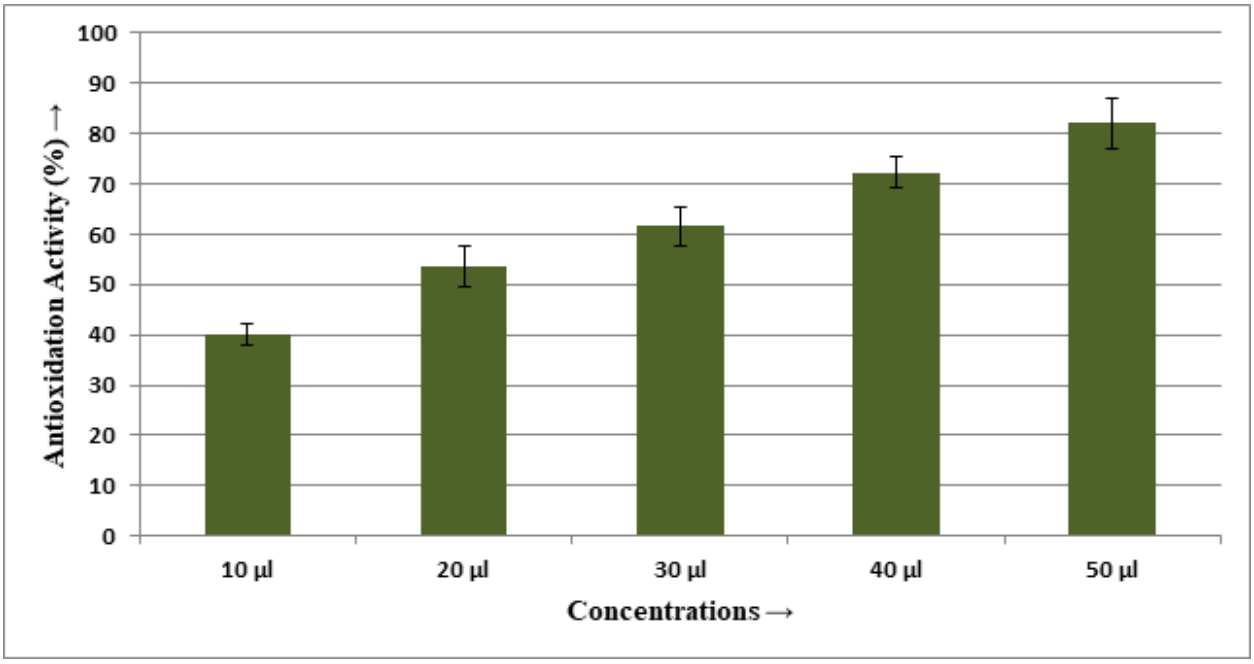

Figure 5: Graphical representation shows the observed Antioxidant Activity (%) in different concentrations of Amla extract

Result of Statistical Analysis

"One –Way ANOVA" refers to one way analysis of variance. Tukey's HSD refers to Tukey's honest significance difference. For the statistical analysis of anti-clastogenecity test five different experimental groups were considered and the statistical results revealed the value of F=24.9 and p=0.01677. As 0.05>p, the null hypothesis had been rejected that there is no significance difference between groups. But when difference within groups were considered then the total number of groups which were significantly different by Tukey's HSD was clearly depicted in Table-6.

Table-6: Shows the statistical analysis result within groups which were significantly different by Tukey's HSDforAnticlastogenecity test.

Sl No	Considered Groups	F value	P value	Remarks from Tukey's HSD
1	Group1- vs- Group4	0.3865	0.0066	As P<0.05; Significantly different
2	Group1- vs- Group5	0.4200	0.0045	As P<0.05; Significantly different
3	Group2- vs- Group4	0.4065	0.0052	As P<0.05; Significantly different
4	Group2- vs- Group5	0..4400	0.0037	As P<0.05; Significantly different
5	Group3- vs- Group4	0.2865	0.0236	As P<0.05; Significantly different
6	Group3- vs- Group5	0.3200	0.0149	As P<0.05; Significantly different

Group-1 – 5, as considered in anticlastogenecity test

Discussion

In the present study the anti-clastogenic and haepato protective properties of crude amla extract were evaluated againsta known clastogen and haepatotoxic agent arsenic. As CA or Chromosomal Aberration study is taken as a sensitive indicator for monitoring genotoxicity of different chemicals, it can be taken as a cytogenetic biomarker. The biochemical assay of liver function parameters like SGPT (Serum Glutamic Pyruvic Transaminase), SGOT (Serum

Glutamic Oxaloacetic Transaminase), Total billirubin and Direct billirubin are used as indicator for hepatotoxicity against various chemicals[20].

In the present study, crude amla extract were kept as a constant concentration against single sodium arsenite dose in both anticlastogenicity and haepato-protective studies. A known clastogenic and antineopastic agent Mitomycin C (MMC) wasusedas positive control in anticlastogenicitystudy and another known hepatotoxic chemical, carbon tetra chloride (CCL_4) was used as positive control in hepatoprotective study whereas the negative control used was the vehicle for all the compounds used.

The total antioxidant activity of the provided sample was evaluated by the green phosphomolybdenum complex formation. This method was mainly based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The colored complex was oxidized by the addition of antioxidant compound causes a reduction in intensity of the blue color[21]. In the present study the crude Amla extract has been shown to have a good antioxidant property in a concentration dependant manner in comparison to ascorbic acid, a known antioxidant.

The incidence of chromosomal aberration in the current study was not significant when crude Amla extract was used alone. The result proved that it did not induce chromosomal aberration indicating its non clastogenic nature. The single sodium arsenitewas able to create a significant amount of chromosomal aberrations in comparison to Mitomycin C (MMC) which proves its clastogenic nature. Pre-treatment of Amla extract before administration of sodium arsenite can reduce the level of chromosomal aberration in a great fashion proving the ameliorative or anti-clastogenic nature of crude Amla extract.

Sodium arsenite, being an arsenic oxoanion has been shown to induce chromosomal aberration in a significant manner. According to Sharma et al 1994, one of the mechanisms suggested for antiinflammatory was due to scavenging effect[22]and similarly in the present study crude extract of amla might had exerted the scavenging activity in preventing the clastogenic effects of Sodium arsenite. According to Yamanak et al. 2004 Sodium arseniteis known to be capable to induce DNA strand breaks in mice often via production of a peroxyl radical [23]and in the present study crude amla extract was found to possess total antioxidant activity in our present work. According to Charoenteeraboon et al 2010 Amla extract had been showntoexer tDPPH (2,2 diphenyl-1-picrylhydrazyl) free radical scavenging activity and ferric reducing antioxidant property [24], so, it can be suggested that in the present study crude amla extract could have exhibited a similar activity to attenuate the nuclear damage induced by the selected clastogen, sodium arsenite.

The increase of liver function parameters in blood serum in the present study was not significant when crude Amla extract was used alone. The result proved that it did not inducehepatotoxicity which indicates its non-hepatotoxic nature. The single sodium arsenite can elevate significant amount of liver function parameters in blood serum in comparison to carbon tetra chloride (CCL_4) which proves its hepatotoxic nature. Pre-treatment of Amla extract before administration of sodium arsenite can reduce the level of liver function parameters in blood serum in a great fashion proving the ameliorative or hepato-protective nature of crude Amla extract.

In liver, hepatic cells contain host of enzymes and perform a variety of metabolic activity. In hepatotoxicity, leakage of the plasma membrane causes the interruption of transport function of liver cells results the release of the enzymes into the blood leading the increased level of these enzymes in serum which is the indication of loss of membrane integrity and cellular leakage. Excessive destruction of heme protein and block of the bile duct within the liver is responsible for the inhibition of conjugation reaction and release of un-conjugated billirubin from the hepatocytes resulting higher concentration of billirubin in serum[25].The possible mechanism of hepatoprotection performed by crude Amla extract was due to the antioxidant property. Due to this particular property, plant extract help to scavenge the reactive oxygen species produced during the metabolism of toxicant and able to show strong membrane stabilization and anti protein denaturation property [26].

The current study showed theresult of crude Amla extract was similar to the previous reports of chemical investigation of Amla which reported that the accumulation of several phytochemicals like flavonoid, steroids, polyphenols, lipids, carbohydrates and vitamin mainly vitamin C were the source of its ameliorative activity against arsenic. It might be possible that the mechanism of anticlastogenic and hepatoprotective effect of crude amla extract was due to its antioxidant property which is particularly for its high content of vitamin C and flavonoid that are well known for potent free radical scavenging activity and enhancement of the antioxidant defense system. The anti-inflammatory effect of steroids can play a significant role in the anticlastogenic and hepatoprotective effect of the extract.

Conclusion

In conclusion the anti clastogenic and hepatoprotective potential of crude amla extract was evaluated against a known toxicant like arsenic. The results showed a positive inhibitory effect of amlaas shown by the frequency of chromosomal aberration in the mouse bone marrow cells *in vivo*. The study also revealed that, amla extract has potential hepatoprotective property in mouse model. The crude amla extract does not show any clastogenic and hepatotoxic activity itself, though it possesses a good antioxidant activity. Amla exert this protection through amelioration by its scavenging property of free radicals and enhancement of the antioxidant defense mechanism. So, in future it could be a possibility for a supplementary alternative or a new phyto derived drug for related human health hazards in arsenic contaminated areas.

Ethical Approval and Consent to Participant Statement

The experiments were followed by the guideline of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals), WBUHS (West Bengal University of Health Science) and approved by Institutional Ethical Committee. The experiment does not used any human data, so consent of participant is not applicable.

Consent for Publication Statement

Not applicable

Availability of Data and Materials Statement

Not applicable

Competing Interests Statements

The authors declare that they have no competing interests.

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Contributorship Statement

Dr. Madhumita J Mukhopadhyay helped for concept, work plan and data summarization. Puspal De performed all the experiments, laboratory work and analysis. All authors approved the final manuscript.

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List of Abbreviation (As they appeared in the text)

1	g	Gram
2	^oC	Degree Celcius
3	CPCSEA	Committee for the Purpose of Control and Supervision of Experiment on Animals
4	WBUHS	West Bengal University of Health Science
5	ml	Millilitre
6	MMC	Mitomycin C
7	$NaAsO_2$	Sodium Arsenite
8	mg	Milligram
9	kg	Kilogram
10	b. w	Body Weight
11	CAE	Crude Amla Extract
12	CCl_4	Carbon Tetrachloride
13	µl	Microlitre
14	mmol	Milli Mole
15	mol	Mole
16	L	Litre
17	dl	Decilitre
18	U/L	Unit Per Litre
19	nm	Nanometre
20	SE	Standard Error
21	ANOVA	Analysis of Variance
22	p	Level of Significance
23	HSD	Honest Significant Differences
24	(+)ve	Positive

25	(-)ve	Negative
26	SD	Standard Deviation
27	SGOT	Serum Glutamic-Oxaloacetic Transaminase
28	SGPT	Serum Glutamic-Pyruvic Transaminase
29	%	Percentage
30	CA	Chromosomal Aberration
31	DPPH	2,2 diphenyl-1-picrylhydrazyl