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Ixora coccinea extract attenuates arsenic-induced cellular dysfunction in HepG2 cells

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ABSTRACT

Arsenic is a metalloid and a well known carcinogen. Chronic exposure to arsenic in drinking water induces oxidative damage to the liver. It is also associated with irrevocable pathological lesions in the liver. In the current study, HepG2 cells were used as a model to understand the cytotoxicity of arsenate and the effect of a standardized extract of *Ixora coccinea* flowers on arsenic-induced liver injury. Reactive oxygen species (ROS), mitochondrial membrane potential (MMP), DNA damage and percentage apoptosis were evaluated in HepG2 cells to understand the cytotoxicity. *Ixora coccinea* is a shrub widely used in Ayurveda and traditional medicinal practices in India and Asia. The flower extract was analysed for free radical scavenging ability using 2,2-diphenylpicrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and nitric oxide scavenging assay. ROS, MMP and DNA damage were considered for assessing the cytoprotective effect of the extract against arsenate-induced cellular damage. IC_{50} value obtained for the cell line was 46 μM. Cellular apoptosis was measured using fluorescent microscopy. Pretreatment of HepG2 cells with *Ixora coccinea* flower aqueous extract significantly ($p < 0.01$) attenuated the ROS levels, altered MMP, apoptosis and DNA damage. Our study demonstrated the role of *Ixora coccinea* flower extract in combating arsenic-induced toxicity in HepG2 cells.

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KEYWORDS: *Ixora coccinea*, HepG2 cells, arsenate, ROS, MMP, DNA damage.

INTRODUCTION

Arsenic (iAs) has been known to cause oxidative damage [1]. Arsenicosis is one of the major health risks associated with the arsenic metalloid [2]. It remains in the environment in several chemical forms with different levels of toxicity. Human exposure to arsenic is mainly through ingestion of arsenic-contaminated water and food. The permissible limit is set to 50 μg/L as per WHO standards. Cancerous and noncancerous effects of arsenic are well documented in exposed subjects [3]. All organs are affected by arsenic; reports indicate that progression of damage in liver tissues is due to reactive oxygen species produced as a result of arsenic exposure [4]. Excessive free radicals produced in the system affect the function of antioxidant defense enzymes. Arsenic-mediated oxidative stress is the cause of fibrinogenesis in liver [5]. The potential role of oxidative stress in arsenic-induced cell injury indicates that a decrease in the concentrations of ROS (reactive oxygen species) may protect hepatic tissue damage in arsenic-exposed subjects. A lot of toxicological investigations are currently concentrated on discovering potent antioxidants.

The pantropical genus *Ixora* is one of the largest genera in Rubiaceae, with more than 530 species of shrubs and small trees that typically grow in the humid rain forest [6]. A large number of plants and phytoconstituents are potent scavengers of excess ROS in fibroblasts [7]. *Ixora coccinea* leaf extracts

are known to reduce intracellular ROS levels. A study indicates the potent inhibitory effects of *Ixora coccinea* on human neutrophil activation and intracellular killing mechanism which contribute to immune cellular mechanism in inflammatory responses [8].

Ixora coccinea belongs to the flavonoid-rich Rubiaceae flowering plant family. *Ixora coccinea* is a shrub and a known folk medicine [9]. The concoction of *Ixora* is mentioned in ancient scripts as a remedy for several ailments. Aerial parts are used to treat ailments such as dysentery, leucorrhoea, dysmenorrhoea, sores, skin diseases, hypertension, chronic ulcers, and some infectious diseases [10, 11]. *Ixora coccinea* extract prepared in hot water is given orally for haemophytis and acute bronchitis [8]. The concoction is prepared by boiling the flowers. It is also used to cure catarrhal bronchitis and dysentery [12]. The flowers contain cycloartenol esters and have cytotoxic, hepatoprotective, antitumor, antimicrobial activity, and wound healing activity [13]. Eczema is treated with shade-dried flowers [14]. The root is used to prepare a concoction, which is effective against nausea, anorexia, and hiccups. Chronic ulcers and sores are healed with the use of root powder [15]. Several studies indicate the cytotoxic, antitumor, and antigenotoxic activity of *Ixora coccinea*. Reports also suggest the wound healing properties of *Ixora coccinea* flowers [16].

Ixora coccinea fruits and flowers are used as dietary supplements [17]. The present study was conducted to estimate the antioxidant capacity of the *Ixora coccinea* extract using DPPH free radical scavenging assays, nitric oxide scavenging assay, ABTS assay, and the efficacy of the plant extract in mitigating the effects of the sodium arsenate-induced effects in HepG2 cells. Intracellular ROS, MMP (Mitochondrial membrane potential), DNA damage, and apoptosis were also evaluated.

MATERIALS AND METHODS

Preparation of samples

Cold maceration of the flower powder was carried out with 1% chloroform. Then the solution was concentrated at the required temperature in a rotary evaporator. Hence obtained extract was used for all experiments.

Determination of total phenols

Total phenol in the flower extract was measured by the following method. Folin-Ciocalteau reagent was used to measure the total phenols in the flower extract. Gallic acid was kept as standard for reference purposes. 5 mL of FC reagent (10 times dilution with water) was mixed thoroughly with 1 mL of extract or standard solution and 700 mM sodium carbonate (4 mL). The absorbance was recorded after the reaction completion in the UV-spectrophotometer at 765 nm [18]. The total phenols were expressed as gallic acid equivalents mg/g of flower dry extract.

Determination of total flavonoid content

The pulversed plant extract was evaluated for its antioxidant activity. The aluminium chloride method was employed to measure the total flavonoids in the flower extract. Quercetin was considered as the standard. 500 microlitre of extract or quercetin was dissolved in 1.5 ml methanol, 10% of 0.1 ml aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml distilled water. This reaction mixture was allowed to stand for about 30 min and the absorbance was read at 415 nm using a photometer [19]. The results obtained from the experiment were expressed as standard equivalents mg/g of the plant extract.

DPPH assay

A free radical scavenging assay was carried out to estimate the antioxidant capacity of the extract. DPPH free radical scavenging assay was used to evaluate the free radical scavenging capability of the *Ixora coccinea* flower extract. 1 ml of 0.1 mM DPPH solution prepared in methanol was mixed with 1.0 ml of the extract at varying concentration (7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL); after 20 min optical density was read at 517 nm with the help of a photometer [20].

ABTS assay

ABTS free radical scavenging assay was carried out to evaluate the antioxidant capacity. ABTS (7 mM) solution was taken along with 2.4 mM potassium persulphate, and this reaction mixture was kept in dark for 15 hours. The flower extract was dissolved in methanol. 180 ml of ABTS was mixed with 20 ml of the test solution. The reaction mixture was allowed to stand for 20 min at room temperature.

The absorbance was recorded at 750 nm in a photometer [21].

Nitric oxide scavenging assay

Griess method was employed to evaluate the antioxidant potential of the extract. Briefly, 2 ml of 10 mM sodium nitroprusside, 0.5 ml of PBS, and 0.5 ml of extract were mixed to obtain the reaction mixture. Different concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL) of the extract were used and allowed to stand at 25 ^{o}C for 90 min. 1 ml sulphanilic acid reagent (0.33% sulphanilic acid in 20% glacial acetic acid) was mixed with 0.5 ml of the reaction mixture. After incubation for 5 min, 1 ml of 0.1% naphthyl ethylene diamine dihydrochloride was added and kept for 30 min. The optical density was read at 540 nm using a photometer [22].

Total antioxidant capacity

The total antioxidant capacity of the extract was measured using the phosphomolybdenum method. 100 µl of standard or extract was mixed with 300 µl of reagent (0.6 M sulfuric acid, 4mM ammonium molybdate with 28 mM sodium phosphate) and allowed to stand at 95 °C for 90 min. After cooling the solution absorbance was read at 695 nm using a photometer. Ascorbic acid was kept as standard and expressed as equivalents of ascorbic acid.

Cell culture

HepG2 cells were procured from NCCS, India. HepG2 cells were grown in a culture flask (Falcon, USA), and incubated in DMEM (High glucose) with FBS 10%, and penicillin-streptomycin 1%, at 37 °C in a CO_2 incubator (NuAire, Plymouth, USA) with 5% CO_2. HepG2 cells were maintained at 85% confluency in T-25 culture flasks and utilized for various assays. The viability of the cells was assessed with the help of a trypan blue dye exclusion assay.

MTT assay

MTT assay was carried out to determine the cytotoxicity of the arsenate. Cells were allowed to attain 85-90% confluency and then used for the assay. HepG2 cells were trypsinized, harvested, and seeded about 1x 10^4 cells per well. Adhered cells were treated with various concentrations of sodium arsenate (2.5, 5, 10, 25, 50, 100 µM), flower extract, and sodium arsenate in combination with *Ixora* flower extract to assess the cytotoxicity. At the end of the experiment formazan crystals formed were dissolved in 0.1 ml of DMSO and optical density was read at 550 nm using the photometer [23].

Estimation of reactive oxygen species

The reactive oxygen species concentration was measured using flow cytometry. Cells were plated at a density of 3x 10^5 in small Petri dishes and were treated with sodium arsenate, extract and blend of extract along with arsenate. The cultures were kept for an hour [24]. The medium was decanted post-incubation, and 5 µM DCFDA was added. Cells were incubated for another 30 min in a 5% CO_2 incubator at 37 °C. HepG2 cells were suspended in PBS after harvesting; the flow cytometer (Becton Dickinson, USA) was used for the analysis.

Evaluation of membrane potential of mitochondria

To estimate MMP, HepG2 cells were placed in 6 cm^2 Petri dishes at a density of 3x10^5. Cells were kept for 24 h with sodium arsenate, flower extract, and the blend of both. After adding the fresh medium, Rhodamine 123 (5 µg/mL) was added. The cells were kept at 37 °C for 30 min in a 5% CO_2 incubator. Trypsinised cells were harvested, washed with phosphate buffer, and centrifuged (1000 rpm) for 10 min [25]. Cells were harvested in cold PBS and read at FACS Calibur (Becton Dickinson) after suspending the cells in 1 ml Chilled PBS.

Comet assay

DNA breaks (Single strand) were enumerated by single-cell gel electrophoresis. HepG2 cells were seeded at a density of 7x10^5 cells in 6 cm^2 plates. HepG2 cells were exposed to sodium arsenate alone, flower extract alone, and in combination (Arsenate and extract) incubated for 24 h. After incubation cells were washed with PBS and suspended in low melting agarose. Roughly 2x10^4 cells were suspended in low melting point agarose. Normal agarose-coated slides were layered with low-melting agarose along with cells maintained at 37 °C and recoated with normal melting agarose. The slides were immersed in the lysing solution maintained at 4 °C overnight; slides were transferred to an electrophoretic chamber containing an alkaline buffer (pH 13). The lysed cells were subjected to electrophoresis in a

horizontal tank for 26 min (300 mA, 20 V). The slides were washed with a neutralizing buffer. Ethidium bromide-stained slides were immediately observed under the fluorescence microscope, photographed, and analyzed using software [26].

Evaluation of apoptotic index

HepG2 cells were seeded in a 6-well plate at a density of $3x10^5$ per well, then kept overnight in a CO_2 incubator at 37 °C, then exposed to the above-mentioned combinations. 0.3 ml of AO/EtBr was added to each well, then the plate was incubated at 37 °C for 30 min. In the end, cells were washed with PBS and observed under the fluorescence microscope for fragmentation and nuclear condensation [25].

Estimation of catalase

HepG2 cells were grown to attain a confluence of 85%. Cells were seeded at a density of 3×10^5 per well in a 6-well plate and kept overnight in an incubator at 37 °C, in 5% CO_2. Cells were treated with arsenate, *Ixora coccinea* extract and in combination, incubated for 24 h; post-incubation, cells were trypsinized and collected in PBS. The harvested cells were subjected to catalase assay [27]. The phosphate buffer (50 mM KH_2PO_4 and 50 mM Na_2HPO_4 in a 1:1.5 v/v ratio) having neutral pH was mixed with H_2O_2 (30 mM) to obtain an absorbance reading of around 0.5 with the help of a photometer. This value was considered as the blank. If the reading was above 0.5 then the volume of buffer in the reaction mixture was increased and if it showed a lower reading then the peroxide content was increased. The volume of the buffer and peroxide was kept constant at 0.9 ml until the end of the experiment. 50 µl of the sample was taken and the experiment was performed.

Estimation of superoxide dismutase

Cells were seeded at a density of 3×10^5 per well in a 6 well plate and kept overnight in an incubator at 37 °C, in 5% CO_2. HepG2 cells were treated with sodium arsenate, *Ixora coccinea* extract, and a combination of arsenate with the extract. Cells were incubated for 24 h, post-incubation cells were trypsinized and collected in PBS. Sodium carbonate (1850 µl) buffer and 50 µl of the sample were mixed. To the reaction mixture, 100 µl of epinephrine was added. The rate of autoxidation of epinephrine to adrenochrome was recorded instantly at 480 nm using a photometer.

Estimation of lipid peroxidation

Lipid peroxidation results in the formation of conjugated dienes, lipid hydroperoxides, and degradation products such as alkanes, aldehydes, and isoprostanes. The most widely used assay to measure lipid peroxidation is the thiobarbituric acid (TBA) assay. The assay is based on the reaction of TBA with bifunctional aldehydes, primarily malondialdehyde (MDA), derived as breakdown products from many oxidized molecules. The resulting chromogen has a characteristic absorbance spectrum with a distinct peak at 532 nm at acid pH.

RESULTS

Total phenols and total flavonoids

The crude extract was analyzed for total phenols and flavonoids. The total phenol found in the extract was 40.11 ± 0.24 mg GAE/g of flower extract. A standard gallic acid calibration curve was used to determine the total phenols with an R^2 value of 0.9975. The total flavonoids estimated were 2.2 ± 0.003 mg QE/g of flower extract. It was determined using a linear calibration curve of quercetin with an R^2 value of 0.9980.

Free radical scavenging assays

DPPH radical scavenging activity was found to be 97.22 ± 3.2 µg/mL. DPPH free radical scavenging assay of the flower extract was found to be more effective than ascorbic acid, a reference standard. The degree of colour change is a measure of the scavenging potential of the antioxidants in the flower extract in terms of hydrogen donating ability. ABTS radical scavenging activity was found to be 71.10 ± 1.8 µg/mL. Ascorbic acid was used as a reference standard in this assay. Flower extract showed more ABTS radical scavenging activity than the reference standard.

Nitric oxide scavenging assay of the extract was found to be 109.15 ± 7.2 µg/mL. Because of its unpaired electron displays important reactivity with different molecules and nitrile free radicals. Antioxidants in the flower extract donate protons to the nitrite radicle; the change in the absorbance was used to measure the nitrite radical scavenging. The flower extract was found to be more effective as nitric oxide free radical scavengers compared to the reference standard curcumin. Total antioxidant capacity was found to be 454 ± 1.12 equivalents/ mg extract µg ascorbic acid.

MTT assay

HepG2 cells were exposed to the flower extract did not show any cytotoxicity. MTT assay was performed to obtain the IC_{50} of sodium arsenate in HepG2 cells i.e. 46 µM for 24 h. The cell survival assay of the HepG2 cells exposed to sodium arsenate (IC_{50}) showed a dose-dependent decrease in cell viability. An increase in percentage cell viability was noted, when the cells were pretreated with *Ixora coccinea* flower extract for 2 h before exposure to sodium arsenate (IC_{50}). The optimal dose was found to be 62.5 µg and was significant ($p < 0.01$) when compared to other concentrations of extract (Fig. 1).

ROS and MMP

Overproduction of ROS would induce oxidative damage to molecules and result in cell death. Dichlorofluroscien (DCF) fluorescence which is indicative of reactive oxygen species levels (Fig. 2a) increased in sodium arsenate (IC_{50})-treated HepG2 cells (358 ± 3.4), which is indicative of reactive oxygen species levels. The cells pretreated with the flower extract showed a significant ($p < 0.01$) decrease in the fluorescence levels (200 ± 2.9). Phenols and flavonoids act as antioxidant agents. These are found in the extract and reduced the ROS levels in cells. Results were significant ($p < 0.05$). Mitochondrial membrane potential (Fig. 2b) collapse was decreased ($p < 0.05$) in cells pretreated with the flower extract, compared to sodium arsenate-treated cells, wherein a significant loss of membrane potential was measured.

DNA damage and apoptotic index

Single strand breaks in the DNA were estimated using alkaline comet assay and expressed as %TDNA (Fig. 3a). Sodium arsenate (IC_{50})-induced DNA damage decreased in pretreated cells significantly ($p < 0.05$). Pretreatment of HepG2 cells with *Ixora coccinea* extract before exposure to 46 µM sodium arsenate caused a decline in the arsenate-induced percentage of tail DNA ($p < 0.05$). The apoptotic index was calculated by counting random 100 cells. Apoptotic execution is followed by chromatin condensation and morphological changes in cells. Cells pretreated with the flower extracts showed a significant decrease in percent apoptosis ($p<0.05$) compared to sodium arsenate-exposed HepG2 cells (Fig. 3b).

SOD, catalase and lipid peroxidation

Superoxide dismutase (SOD) and catalase activity increased significantly ($p < 0.05$) in cells pretreated with an optimal dose of *Ixora coccinea* extract (Fig. 4) compared to sodium arsenate-treated cells (IC_{50}).

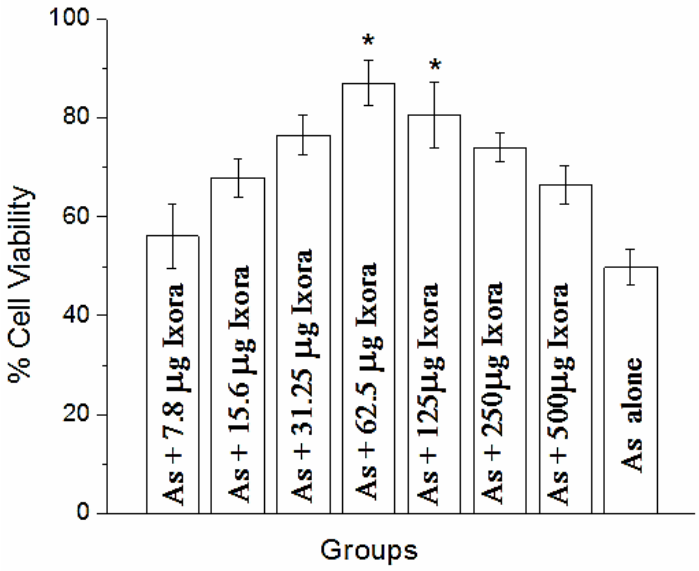

Fig. 1. Evaluation of the alterations in cell viability of HepG2 cells treated with different concentrations of *Ixora coccinea* extract and arsenate (As) using MTT assay. *$p < 0.05$ compared to the arsenate alone-treated group.

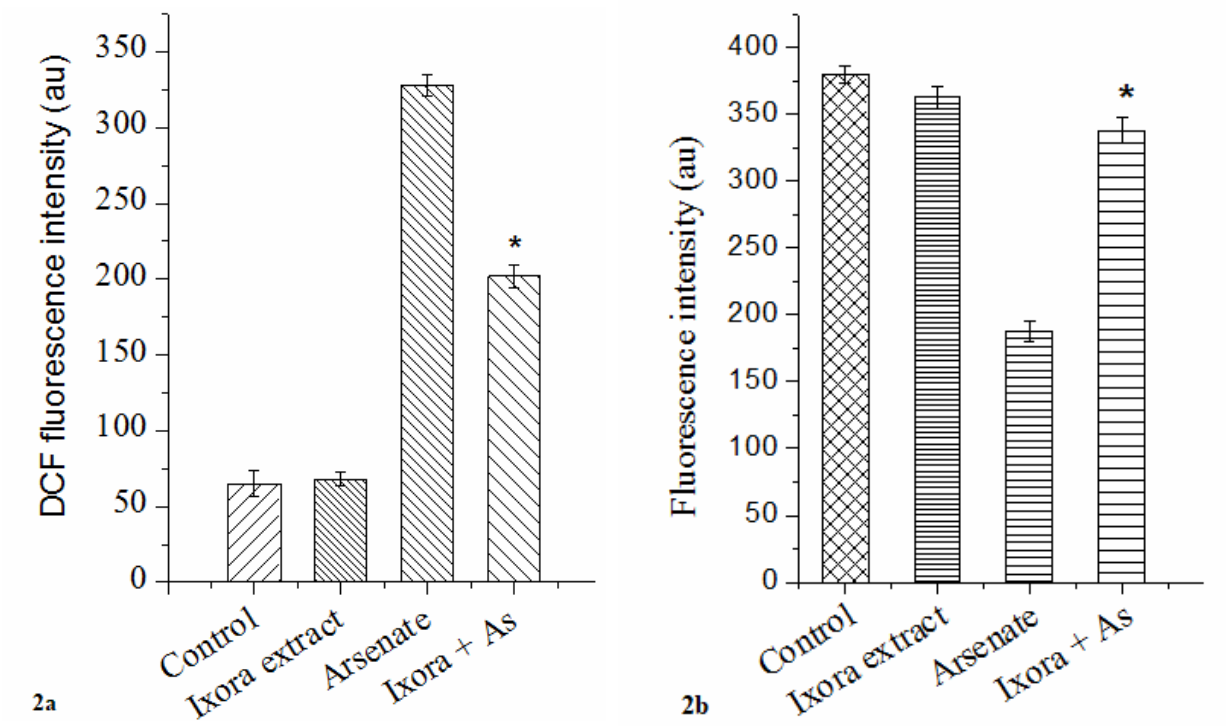

Fig. 2a. Intracellular ROS after treating HepG2 cells with *Ixora coccinea* extract, arsenate and their combination. **2b.** Restoration of mitochondrial membrane potential after treating HepG2 cells with *Ixora coccinea* extract, arsenate and their combination. *p < 0.05 compared to the arsenate-treated group.

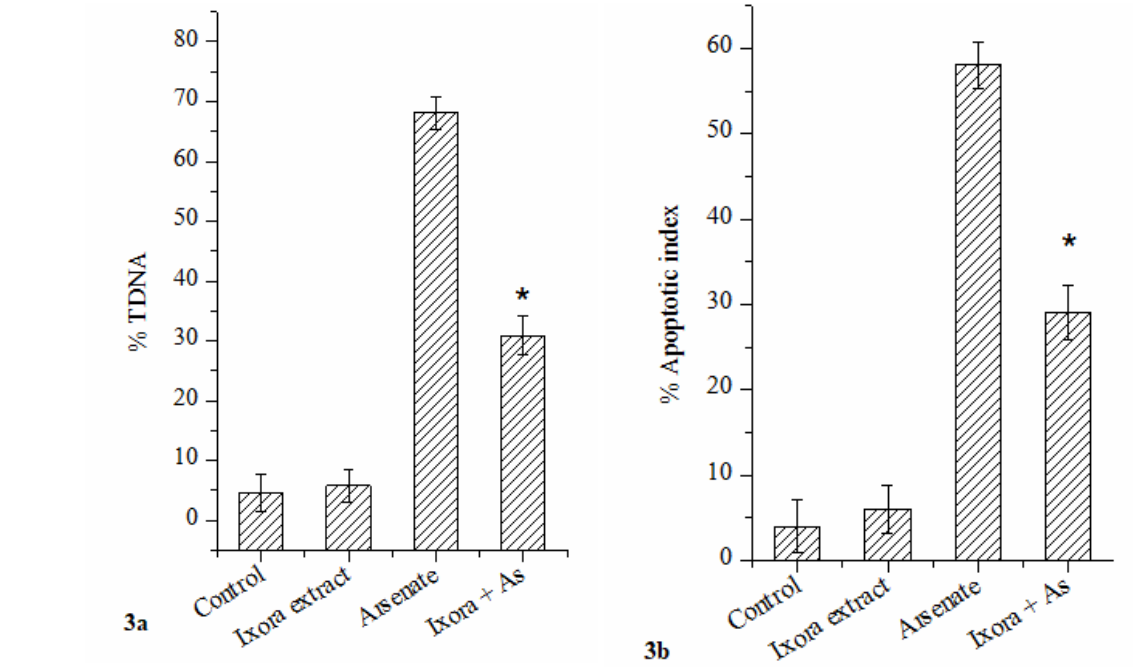

Fig. 3a. Altered DNA damage after treating HepG2 cells with *Ixora coccinea* extract, arsenate and their combination. Significance (p < 0.01). **3b.** Percentage apoptotic index after treating HepG2 cells with *Ixora coccinea* extract, arsenate and their combination. *p < 0.05 compared to the arsenate-treated group.

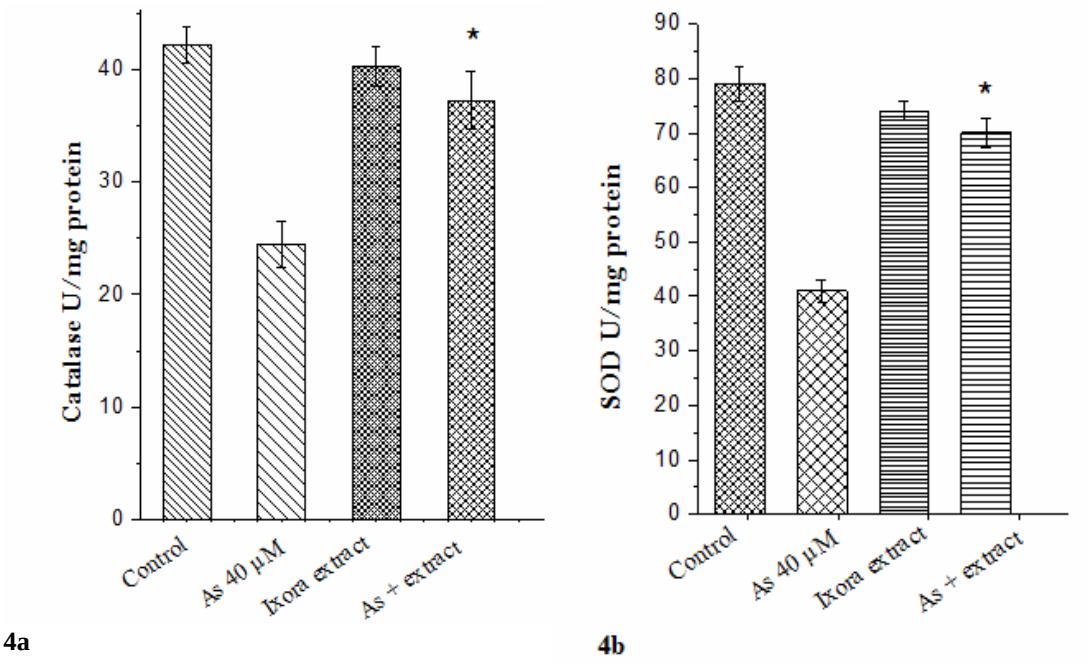

Fig. 4. catalase activity (**a**), and SOD activity **(b)** after treating HepG2 cells with *Ixora coccinea* extract (62.5 µg/ml), arsenate (40 µM) and in combination. Results were significant ($p < 0.05$). *$p < 0.05$ compared to the arsenate-treated group.

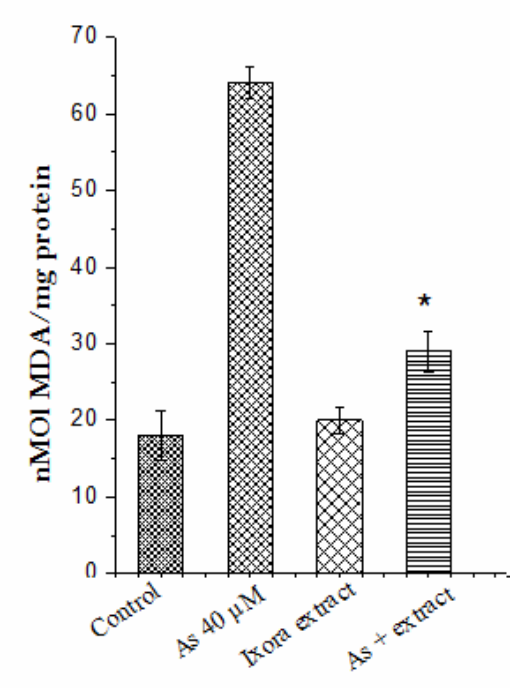

Fig. 5. Lipid peroxidation decreased significantly in *Ixora coccinea*-treated HepG2 cells compared to arsenate-treated group ($p < 0.05$). *$p < 0.05$ compared to the arsenate-treated group.

Antioxidant enzymes are required for keeping the intrinsic balance of reactive oxygen species and antioxidant system. ROS often leads to the peroxidation of membrane lipids. Arsenate is known to induce lipid peroxidation. Lipid peroxidation (Fig. 5) also decreased significantly in pretreated cells compared to the group which received sodium arsenate (IC_{50}) ($p < 0.05$).

DISCUSSION

Arsenic is an environmental contaminant that causes significant health issues for populations with chronic exposure. It exerts its toxicity by adversely affecting different biochemical processes in the body. The liver is one of the major organs responding to environmental toxins [28]. The importance of natural antioxidants has increased during recent years owing to their numerous pharmacological properties. It is well known that nutraceuticals from extracts of medicinal plants can modulate physiological functions and thereby provide beneficial effects against various diseases. Arsenicosis therapy may include the identification and characterization of therapeutic agents from Phyto-constituents.

Ixora coccinea flower extract was analyzed for phytochemical constituents and for its free radical scavenging capacity. The plant extract consists of polyphenols and flavonoids known to have several pharmacological properties. The free radical quenching ability could be attributed to abundant polyphenols and flavonoids. The edible plants in our diet are known to lower the toxic effects of toxicants in the environment [29].

Phenols are well known reducing agents, free radical scavengers, and metal chelators. Phenols are a major class of antioxidants due to their chemical structure. There are also reports of flavonoids being scavengers of free radicals possessing antioxidant properties. Phenolic compounds and flavonoids were measured in *Ixora coccinea* flower extract. ABTS scavenging is due to the presence of polyphenolic compounds. Phosphomolybdynum complex formation was evident in the extract indicating the total antioxidant activity. In the current study, the ameliorating ability of the flower extract was analyzed against arsenic-induced cytotoxicity.

Reactive nitrogen species also play an important role in metal-induced toxicity causing oxidative stress and DNA damage [30]. The enhanced generation of superoxide in the presence of equimolar concentrations of nitric oxide will lead to the formation of the potent oxidant and nitrating agent peroxynitrite [31]. The current study demonstrated that *Ixora coccinea* aqueous extracts possess nitric oxide scavenging capacity and other free radical scavenging ability.

Chronic arsenic exposure induces hepatic oxidative stress due to increased ROS generation. Reactive oxygen species consists of species such as superoxide anion, hydrogen peroxide, and hydroxyl-free radical [32]. Experimental results also indicate the generation of superoxide ion and peroxide in arsenic-exposed cell lines such as smooth muscle cells, endothelial cells, and keratinocytes. *Ixora* species is known to have free radical scavenging and ROS reducing ability. The high concentration of ROS in cells induces lipid peroxidation and oxidative stress. ROS accumulation inside the cells increases the mitochondrial hydrogen peroxide production and lipid peroxidation of the mitochondrial membrane, resulting in loss of membrane integrity and stability leading to apoptosis [33]. Results from the current study showed that pretreatment of HepG2 cells with *Ixora coccinea* resulted in a decrease in ROS levels induced by arsenate and the flower extract was potent enough to protect the cell from damage. Scavenging the excessive ROS through the antioxidant defense system is critical for the maintenance of the intrinsic redox balance and protects cells from oxidative injury after arsenic exposure.

Excess ROS and mitochondrial dysfunction are linked. The mitochondrial electron transport system is vulnerable to arsenic toxicity which causes a decrement in ATP production, suppresses mitochondrial transmembrane potential, and augments ROS generation [34]. Arsenate-induced shift in mitochondrial membrane potential restoration was exhibited by the extract. Mitochondrial damage and oxidative stress cause apoptosis when exposed to arsenate.

Arsenic induces stress in the biological system; hence, it is known to result in autophagy *via* ROS-dependent pathway [35]. Arsenate-induced percent apoptosis was reduced significantly as a result of quenching of ROS by the polyphenols and flavonoids present in the extract indicating the mitoprotective capacity.

Exposure to toxicants may result in either partial damage to cellular functions or cell death, depending on the type of assault, the type of cells, and their internal defense mechanisms [36]. The cellular enzymatic antioxidants like superoxide dismutase (SOD) and catalase play an important role. O_2^- is subsequently dismutated into hydrogen peroxide (H_2O_2) by SOD enzyme. Reports are suggesting a decrease in SOD, catalase activity along with citric acid cycle-related enzyme activity in cells and animal models exposed to arsenic compounds [35]. In the current study, it was shown that phytochemicals elevated the SOD and catalase activity in arsenate-exposed HepG2 cells.

Polyphenols and flavonoids quench the ROS thus protecting the cells from DNA damage. Phenolic compounds are known to suppress apoptosis and DNA fragmentation. There was a significant increase in DNA damage and fragmentation in the cell population exposed to arsenate. *Ixora* extract reduced DNA fragmentation and percent apoptosis in cells exposed to arsenate exhibiting the antigenotoxic effect. Naturally available antioxidants

are useful in preventing arsenic-induced oxidative damage to the cells [37]. Edible plants have been documented to reverse the effect of arsenic-induced toxicity. *Ixora coccinea* flower extract possesses ameliorative properties because of the presence of phenolics, and flavonoids. Further studies have to be realized to understand the transcriptional activation of genes coding for antioxidant repertoire by binding to the antioxidants.

CONCLUSION

The results of the studies corroborate that *Ixora coccinea* flower extract can be used to reduce the arsenic-induced toxicity. The experiments were designed to understand the attenuation of sodium arsenate-induced toxicity by *Ixora coccinea* extract in HepG2 cells. The ameliorative ability of the flower extract against arsenic-induced toxicity may be attributed to its anti-genotoxic, anti-apoptotic, and anti-lipid peroxidative potential plausibly because of its free radical scavenging ability of polyphenols and flavonoids. Arsenic exposure is known to affect the antioxidant enzymes and hence affect the cellular function, and *Ixora coccinea* extract alleviated the toxicological effects. These results open up novel research areas towards the implementation of natural therapy to reverse the arsenate-induced cytotoxicity in the liver, as the liver is a major organ involved in metabolism. The aspect of nutrition, pollution, and arsenic interaction in living beings has to be realized.

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CONFLICT OF INTEREST STATEMENT

None.

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