RESEARCH ARTICLE

Nanocurcumin Prevents Oxidative Stress Induced following Arsenic and Fluoride Co-exposure in Rats

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ABSTRACT

The present study is in continuation of our previous efforts to investigate the preventive efficacy of encapsulated curcumin nanoparticles (nanocurcumin) in mitigating effects of arsenic and/or fluoride. Curcumin was encapsulated in chitosan nanoparticles having a size distribution in the range of 50 nm. Sodium arsenite (2 mg/kg, orally) and Fluoride (50 ppm in drinking water) either alone or in combination were administered to male Wistar rats for four weeks to evaluate the efficacy of nanocurcumin (15 mg/kg) in rats. The preventive efficacy of nanocurcumin was evaluated against various altered biochemical variables suggestive of oxidative stress in liver and kidneys, and concentration of As and F in blood. Nanocurcumin co-administration with arsenic and fluoride resulted in lowering of reactive oxygen species and restoration of blood glutathione level which were found to be altered in arsenic and fluoride intoxicated groups. Nanocurcumin were also found to be effective in reversing δ-aminolevulinic acid dehydratase (ALAD) inhibition caused by arsenic exposure. The most promising result from our study shows that nanocurcumin removes not only arsenic but also fluoride from blood which may be due to the enhanced bioavailability and moderate chelating potential of nanocurcumin.

Keywords: Nanoencapsulation, curcumin, arsenic-fluoride co-exposure, oxidative stress, preventive effects

1. INTRODUCTION

Arsenic (As) and fluoride (F) are potent toxicants, widely distributed through drinking water and food and often result in adverse health effects. Millions of people are currently at risk all over the world because they drink water containing carcinogenic amounts of As and F. Permissible limit of As in water is 10 μg/l and for F it is 1 mg/l as per WHO guidelines[1]. The occurrence of As and F in groundwater has been widely reported in Latin America, especially in arid and semi-arid regions of Mexico, Argentina, and Chile[2]. Few reports also suggest the coexistence of the two toxicants in other areas of the world including, India[3]. The interaction of As with F could be a key in the complete elucidation of the molecular mechanisms involved in the development of inflammatory and malignant diseases[4]. Zhang[5], *et al.* suggested that the toxicological effects of fluoride could be enhanced by arsenic while, contradictory results suggesting independent, synergistic and antagonistic effects in different animal models too have been reported[6-10]. It has been also suggested that arsenic–fluoride interaction during co-exposure induces oxidative stress and DNA damage[11-13]. The potential role of oxidative stress in the cell injury associated with arsenic and fluoride poisoning suggests that antioxidants can be employed in the treatment protocols to mitigate arsenic- and fluoride

induced toxicity. Vitamin E[10], melatonin[14] and curcumin[15] supplementation during co-exposure has been found to be beneficial in restoring altered biochemical variables, maintaining pro-oxidant/antioxidant balance, and reducing genotoxicity as well as body arsenic and fluoride levels.

Among other strategies emerging to counteract metal toxicity is site-specific targeted delivery of drugs using appropriate carriers such as liposomes, micelles and nanoparticles. Nanoparticles due to its ability to evade reticuloendothelial system (RES) such as liver Kupffer cells and spleen macrophages can be delivered to the desired site effectively. Another advantage of using nanoparticles for drug delivery is its potential to cross blood-brain barrier; which further enhances its specific delivery. We have recently reported a novel strategy using encapsulated curcumin nanoparticles to counteract arsenic toxicity[16]. Our hypothesis was based on the fact that nanoencapsulation of curcumin would result in increasing its bioavailability and thus would be an interesting strategy to reduce the dose required for a desired effect against metal toxicity. More recently, nanocurcumin synthesised and characterised by our group have been shown to be effective against lead induced oxidative stress[17].

In continuation of our efforts to design an effective strategy for metal toxicity, the present study was planned to assess the preventive effects of nanocurcumin on toxic effects of arsenic and fluoride individually, and during co-

exposure on biochemical variables suggestive of alterations in hematopoietic, hepatic and renal oxidative stress, and arsenic and fluoride concentration in blood.

2. METHODS

2.1 Chemicals and Reagents

Curcumin, chitosan (molecular weight 400 kDa), and sodium meta-arsenite ($NaAsO_2$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade and purchased from Sigma-Aldrich, Merck (Darmstadt, Germany) or BDH Chemicals (Bengaluru, India). Triple-distilled water was prepared using a Millipore (Billerica, MA, USA) water purification system and was used throughout the study.

2.2 Synthesis and Characterisation of Encapsulated Curcumin Nanoparticles

Nanocurcumin were synthesised and characterised as reported previously[16]. Briefly, Tween 80 (0.5 ml) was added to 20 ml of a 0.15% solution of chitosan in dilute acetic acid (35 mM) with constant stirring for 1 hour. Then 250 µl of curcumin solution (10 or 20 mg/ml in chloroform) was added in aliquots of ∼ 20 µl with stirring. The solution was stirred for a further 1 hour, after which 0.5 ml of 20% sodium sulfate solution was added drop wise with stirring and stirring was continued for another 30 minutes. To crosslink the nanoparticles, 0.1 ml of glutaraldehyde was added to the solution and stirring continued for another 30 minutes. Finally, 1 ml of 10% sodium metabisulfite was added to the solution and stirred for another 30 minutes. The solution was left to stand for 12 hours and was then dialysed against water with two changes at 4 °C for 24 hours, and then against normal saline with two changes at 4 °C for 24 hours. The dialysed solution was preserved at 4 °C for characterisation and efficacy studies. The solution was lyophilised to yield a thick, viscous liquid. The size and morphology of the nanoparticles were determined by transmission electron microscopy (TEM) using a JEOL JEM 2000EX200 electron microscope.

2.3 Efficacy of Nanocurcumin: Animal exposure

An experiment was designed to evaluate the preventive efficacy which included the antioxidant and metal chelating property of nanocurcumin when administered by oral route against chronic arsenic and fluoride exposure in male Wistar rats.

Animals were obtained from the animal house facility of the Defense Research and Development Establishment (DRDE), Gwalior. The animal ethics committee of DRDE approved the experimental protocol. Experiments were performed on male Wistar rats (150±15 g), housed in stainless steel cages in an air-conditioned room maintained at 25±2°C and 12 hour light: 12 hour dark cycles. The animals were acclimatised for 7 days prior to their use in experiments and were allowed a standard diet and water throughout the study.

The rats were divided into seven groups of six rats each so that the group mean and standard deviation in body weight were approximately equal. Animals were treated for 4 weeks according to the following protocols:

Group 1: normal (no treatment)
Group 2: arsenic (sodium arsenite, 2 mg/kg orally for 4 weeks)
Group 3: fluoride (50 ppm in drinking water for 4 weeks)
Group 4: arsenic + fluoride (same as in group 2 and 3)
Group 5: arsenic + nanocurcumin (15 mg/kg orally for 4 weeks)
Group 6: fluoride + nanocurcumin (15 mg/kg orally for 4 weeks)
Group 7: arsenic + fluoride + nanocurcumin (15 mg/kg orally for 4 weeks)
Group 8: nanocurcumin (15 mg/kg orally for 4 weeks)

The dose for nanocurcumin was chosen based on our previously reported study[16-17]. For simplicity purposes in the experiment we have not kept the bulk curcumin group. After 4 weeks, rats were anesthetised under light ether and blood samples were collected using a needle via intra-cardiac puncture. The animals were sacrificed and samples of brain and liver were collected for biochemical and arsenic analyses.

2.4 Biochemical Analysis

2.4.1 ROS assay

ROS levels in blood, brain and liver were measured using 2′,7′-dichlrofluorescein diacetate (DCFDA), which is converted to highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The assay was performed as described by Socci[18], *et al.* In brief, 5% RBC hemolysate and 1% brain and 1% liver homogenates were prepared in ice-cold 40 mM Tris–HCl buffer (pH 7.4). Samples were further diluted to 0.25% with the same buffer and placed on ice. Each sample was divided into two equal fractions (2 ml each). To one fraction, 40 µl of 1.25 mM DCFDA in methanol was added for ROS estimation. The same volume of methanol was added to the other fraction as a control for sample auto fluorescence. All samples were incubated for 15 minutes in a 37°C water bath. The fluorescence was determined at 488 nm excitation and 525 nm emission using a Perkin-Elmer (UK) LS-55 fluorimeter. Blood, brain and liver ROS readings are expressed as arbitrary fluorescence intensity units (FIU at 530 nm).

2.4.2 Glutathione (GSH) Assays

Blood GSH concentrations were assayed using a slight modification of a literature method.[19] Approximately 0.2 ml of whole blood was added to 1.8 ml of distilled water and incubated for 10 min at 37 °C for complete hemolysis. After adding 3 ml of 4% sulfosalicylic acid, the tube was centrifuged at 2500 rpm for 15 minutes. To the supernatant, 0.2 ml of 10 mM 5,5′dithiobis-(2 nitrobenzoic acid) was added in the presence of phosphate buffer (0.1 M, pH 7.4). The absorbance at 412 nm was used to calculate the GSH concentration.

Brain and liver GSH and GSSG were estimated as previously described[20]. In brief, 0.25 g of tissue sample was homogenised on ice with 3.75 ml of 0.1 M phosphate–0.005

M EDTA buffer (pH 8.0) and 1 ml of 25% HPO_3 as a protein precipitant. The homogenate (4.7 ml) was centrifuged at 100,000g for 30 minutes at 4°C. For the GSH assay, 0.5 ml of supernatant and 4.5 ml of phosphate buffer (pH 8.0) were mixed. The final assay mixture (2.0 ml) contained 100 µl of supernatant, 1.8 ml of phosphate–EDTA buffer and 100 µl of *o*-phthalaldehyde (OPT; 1000 µl/ml in absolute methanol, prepared fresh). After mixing, the fluorescence was determined at 420 nm with an excitation wavelength of 350 nm using a Perkin-Elmer LS-55 fluorimeter.

For the GSSG assay, 0.5 ml of supernatant was incubated at room temperature with 200 µl of 0.04 M *N*-ethylmaleimide for 30 min. To this mixture, 4.3 ml of 0.1 M NaOH was added. An aliquot of 100µl of this mixture was used to measure GSSG according to the procedure described above for GSH, except that 0.1 M NaOH was used as the diluent instead of phosphate buffer.

2.4.3 *Blood δ-aminolevulinic Acid Dehydratase (δ-ALAD) Activity*

Blood δ-ALAD was assayed according to the method of Berlin and Schaller[21]. The assay system consisted of 0.2 ml of heparinised blood and 1.3 ml of distilled water. After 10 minutes of incubation at 37 °C for complete hemolysis, 1 ml of standard δ-ALA was added to the tube and incubated for 60 minutes at 37 °C, when the reaction was stopped by adding 1 ml of trichloroacetic acid. An equal volume of Ehrlich's reagent was added to the supernatant and the absorbance was measured at 555 nm after 5 min.

2.4.4 *Glutathione peroxidase (GPx) activity*

Glutathione peroxidase enzyme is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. Glutathione peroxidase activity was measured by the literature procedure[22]. The activity of GPx was determined by quantifying the rate of H_2O_2-induced oxidation of reduced glutathione (GSH) to oxidised glutathione (GSSH). A yellow product, which had absorbance at 412 nm, could be formed as GSH reacted with dithiobisnitrobenzoic acid. The reaction involved measure of the reduced GSH. 5,5'-dithiobis-2-nitrobenzoic acid is reduced by free –SH groups of glutathione to form 1 molecule of 5-thio-2-nitrobenzoic acid per molecule of –SH. The nitromercaptobenzoic acid anion released has an intense yellow color and can be used to measure –SH groups.

Briefly, supernatant obtained after centrifuging 5% tissue homogenate at 1500×g for 10 minutes followed by 10,000×g for 30 minutes at 4 °C was used for GPx assay. 1 ml of reaction mixture was added to 0.3 ml of tissue homogenate supernatant. After incubation at 37 °C for 15 minutes, reaction was terminated by addition of 0.5 ml TCA. Tubes were centrifuged at 1500×g for 5 minutes and supernatant was collected. 0.2 ml of phosphate buffer (0.1M pH 7.4) and 0.7 ml of DTNB was added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm. The activity of enzyme was expressed as µg of GSH consumed/min/mg protein.

2.4.5 *Glutathione-S-transferase (GST) Activity*

GST activity was determined following the literature procedure[23]. The GST activity is determined by measuring the rate of formation of conjugate of GSH and 1-chloro-2, 4-dinitrobenzene (CDNB), which in turn is determined spectrophotometrically by recording increase in absorbance at 340 nm.

Briefly, reaction mixture contained 0.02 ml of 1-chloro 2, 4-dinitro benzene and 2.9 ml of GSH and 30 µl of post mitochondrial supernatant and change in color were monitored by recording absorbance (340 nm) at 30 sec intervals for 3 minutes. The activity of enzyme was expressed as nmoles of GSH-CDNB conjugate formed/min/mg protein, using molar extinction coefficient of the conjugate (9.6×106 $M^{-1}cm^{-1}$).

2.4.6 *Thiobarbituric Acid Reactive Substances Assay*

Thiobarbituric acid reactive substances (TBARS) were measured according to a method reported in literature[24] by incubating whole-tissue homogenate with 8.1% SDS (w/v) for 10 minutes, followed by addition of 20% acetic acid (pH 3.5)[23]. The reaction mixture was further incubated with 0.6% TBA (w/v) for 1 hour in a boiling water bath. The pink chromogen that formed was extracted in butanol pyridine solution and the absorbance was measured at 532 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56×10^5 M/cm.

2.5 Liver and Kidney Function Tests

GOT and GPT as liver function test (LFT) and urea and creatinine as kidney function test (KFT) were done by an automatic clinical analyser (Erba Mannheim, EM200) according to the manufacturer's protocol. Reagents for the assays were provided by the same manufacturer.

2.6 Urinary 8-OHdG levels

8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in urine were measured using commercial kits (8-OHdG, highly sensitive 8-OHdG check kit, JAICA, Fukuroi, Japan) by the protocol provided by the manufacturer.

2.6 Arsenic and Fluoride Estimation in Blood

As concentration in blood was measured following wet acid digestion using a microwave digestion system (Anton Paar Multiwave 3000, Austria, Europe). Samples were brought to a constant volume, and determination was performed using an auto sampler (As-72) and graphite furnace (MHS) fitted with an atomic absorption spectrophotometer (AAS, Perkin-Elmer model AAnalyst 100). A calibration curve was constructed by adding known amounts of arsenic standard (TraceCERT®, 1000 mg/L As in nitric acid, Sigma-Aldrich) to calculate arsenic levels.

F concentration was measured in the digested blood and tissue samples using Orion ion analyser (Orion, Seattle, WA, USA) as reported previously[9]. Briefly, by constructing the cell using the fluoride ion-selective electrode and calomel reference electrode in a solution of fluoride at pH 5.35 adjusted with total ionic strength adjusting buffer

(TISAB), the cell potential was determined. By measuring the cell potential for a series of fluoride standards like 0.1, 1.0, 10.0, 100, and 1000 mg/L and constructing the standard calibration graph by plotting the cell potential versus log(F), it is possible to find out the unknown fluoride concentration from measured cell potential. The detection limit of the instrument was 0.025 mg/L to 500 mg/L.

2.7 Statistical Analysis

Results are expressed as mean ± SEM for the number of observations indicated. Mean values were compared using one-way analysis of variance (ANOVA) followed by Bonferroni's test to compare means between the different treatment groups. Differences were considered significant at $p < 0.05$ unless otherwise stated.

3. RESULTS

3.1 Nanocurcumin Synthesis and Characterisation

Curcumin was encapsulated in chitosan nanoparticles cross-linked with glutaraldehyde as reported previously[16]. The size and size distribution of the nanoparticles demonstrated the particles have spherical morphology and low polydispersity, with an approximate size of ∼ 50 nm (Fig. 1).
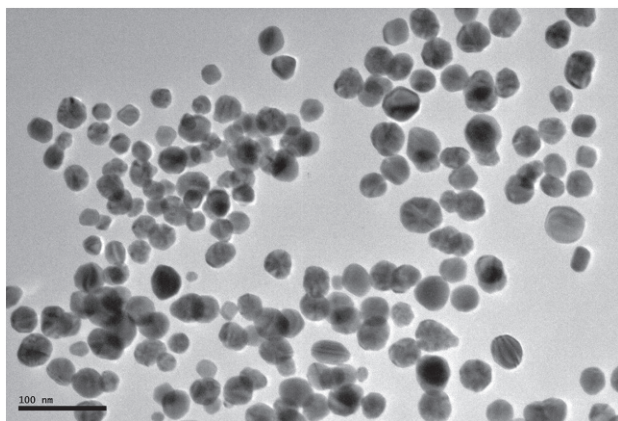
Figure 1. Size characterisation of the nanoparticles using transmission electron micrograph studies.

3.2 Body Weight Changes

Effect of exposure to arsenic, fluoride and nanocurcumin either individually or in combination on body weight gain in rats is shown in Table 1. The difference in body weight at the end of exposure was not statistically different compared to control animals.

3.3 Oxidative Stress Parameters in Blood

Figure 2 indicates toxic effects of arsenic and fluoride on some blood biochemical variables followed by significant protection with nanocurcumin. Individual and combined exposure to arsenic and fluoride significantly induced the elevated level of ROS followed by depleted GSH level suggestive of oxidative stress. δ-ALAD activity also found to be inhibited in the groups exposed with arsenic alone or in combination with fluoride. On the other hand, concomitant

Table 1. Individual and combined effect of As, F and NC on body weight gain in rats

Treatment	Body Weight Gain (g)
Control	87.0±5.6
As	88.8±2.2
F	100.4±3.4
As + F	81.0±4.9
As + NC	81.6±1.9
F + NC	82.4±4.2
As + F + NC	69.0±4.0
NC	70.2±4.2

Abbreviations used and units: Body weight in grams. Values are mean ± SEM; (n = 5).
Differences between values with matching symbol notations within each row are not statistically significant at a 5% level of probability.
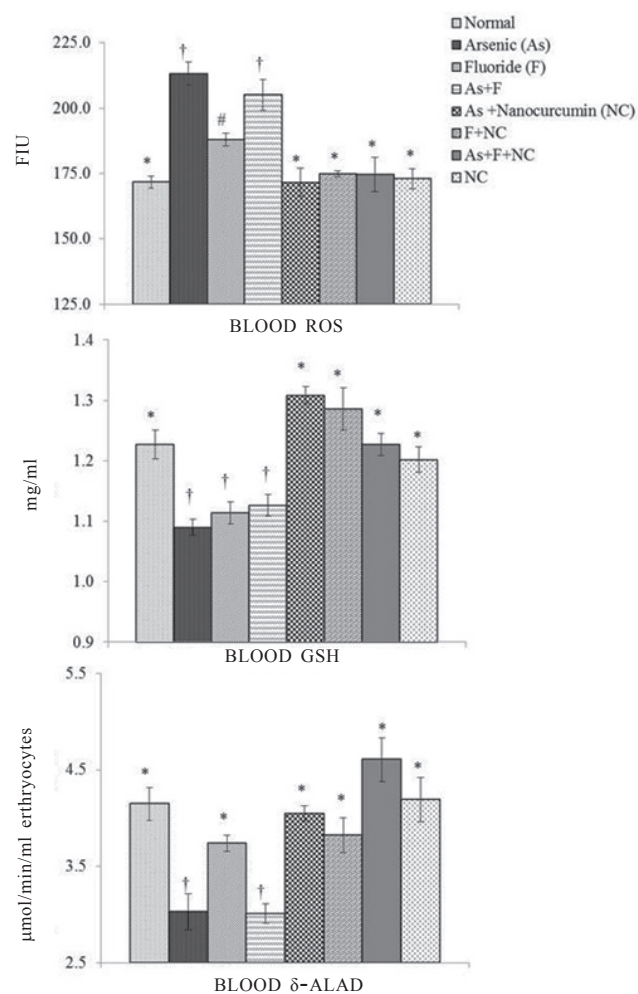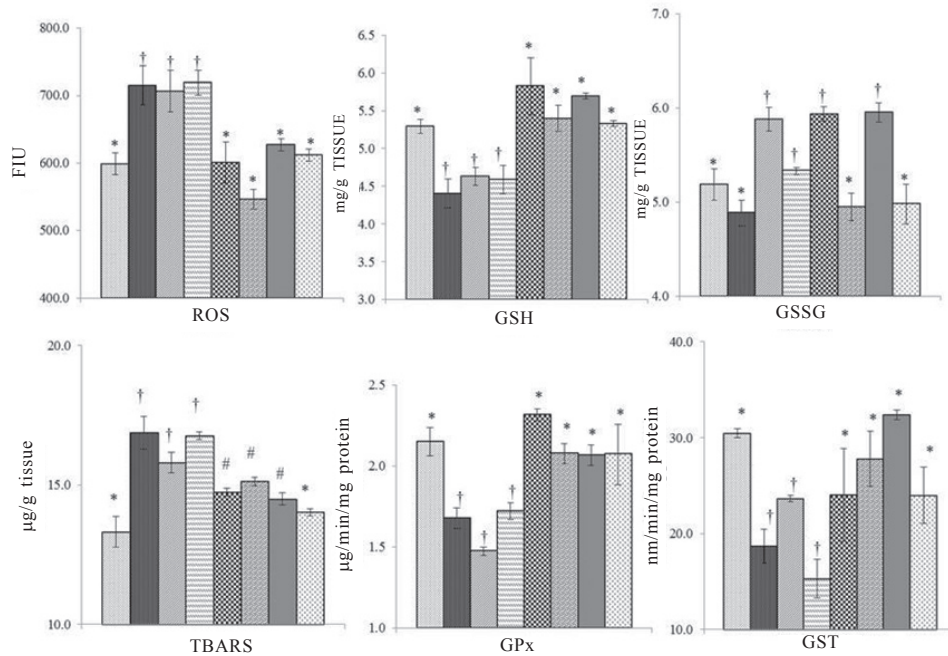

Figure 2. Effect of nanocurcumin supplementation on arsenic and fluoride induced altered blood biochemical variables in rats. Values are mean ± SEM, *n* = 5. Values with the same symbol within each bar are not significantly different at the 5% level of probability.

nanocurcumin treatment significantly showed protection in all the altered blood biochemical variables compared to the exposed animals.

3.4 Hepatic and Renal Oxidative Stress Variables

Figure 3 and 4 show protective efficacy of nanocurcumin on altered tissue ROS, GSH, TBARS, GPx and GST levels induced by individual and combined exposure to arsenic and fluoride and its concomitant treatment with nanocurcumin. The graph shows that exposure to arsenic and fluoride led to a significant increase in ROS level whether given alone or in combination. On the other hand, exposure to arsenic and fluoride reduced the levels of GSH in both the tissues of rats. TBARS level was found to be increased significantly on exposure to arsenic and fluoride alone and in combination. Exposure to arsenic and fluoride also altered levels of glutathione linked enzymes, GPx and GST. GPx and GST activities were reduced in both liver and kidney when given alone or in combination. Whereas, nanocurcumin co-treatment significantly protects the

Figure 3. Effect of nanocurcumin supplementation on arsenic and fluoride induced altered hepatic biochemical variables in rats. Values are mean ± SEM, *n* = 5. Values with the same symbol within each bar are not significantly different at the 5% level of probability.
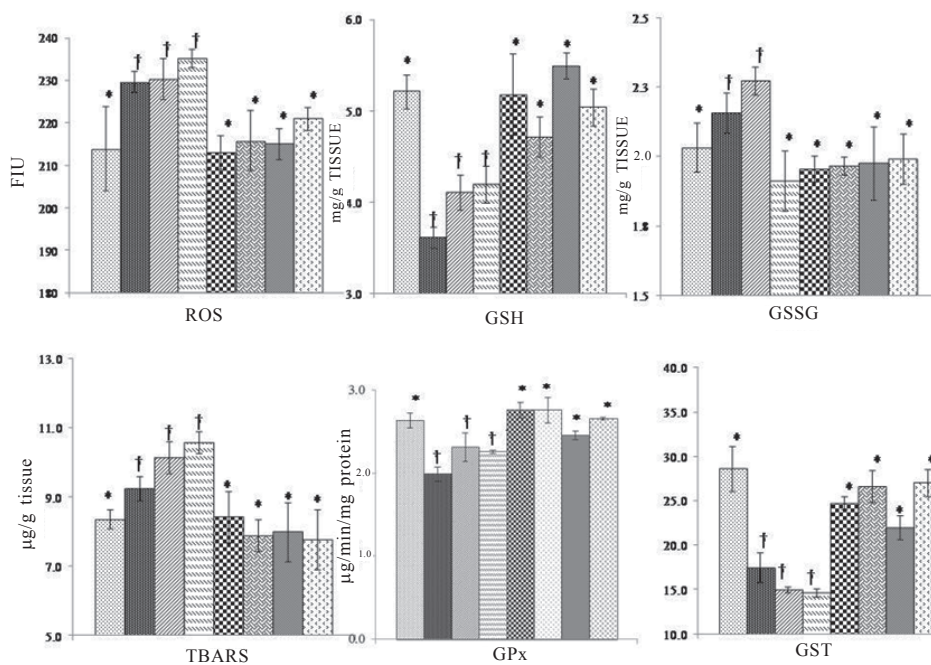
Figure 4. Effect of nanocurcumin supplementation on arsenic and fluoride induced altered renal biochemical variables in rats. Values are mean ± SEM, *n* = 5. Values with the same symbol within each bar are not significantly different at the 5% level of probability.

elevated level of ROS and restores GSH levels in both liver and kidney and bring it towards the normal level. However, prominent protection in GPx level was observed in arsenic with nanocurcumin treated group while GST protection was more pronounced in As+ F+ NC combination group in liver.

3.5 Liver and Kidney Function Tests in Plasma

Table 2 shows the effect of arsenic and fluoride exposure with co-supplementation of nanocurcumin on liver and kidney function tests. Liver is an important target for As toxicity and the same is reflected by significant differences in LFT parameters (GOT, GPT) on exposure to arsenic as compared to control. Nanocurcumin was slightly effective as observed in restoration of GPT levels in As+NC exposed group. However, no significant change was observed in other parameters.

Table 2. Effect of nanocurcumin supplementation on arsenic and fluoride induced altered SGPT, SGOT, Urea and Creatinine activities in blood

	GPT	GOT	Urea	Creatinine
Control	95.6±2.9*	139.1±4.1*	39.8±2.1*	0.60±0.01*
As	70.3±3.5†	124.3±1.4†	36.7±3.3*	0.64±0.02†
F	75.9±6.6†	138.5±4.5*	46.3±2.8*	0.64±0.02†
As + F	76.4±2.7†	126.5±3.6†	44.2±1.6†	0.65±0.01†
As + NC	82.1±2.6†	144.9±7.0*	38.4±1.5*	0.65±0.02†
F + NC	76.5±4.3†	140.1±4.7*	43.1±2.6†	0.66±0.02†
As + F + NC	71.8±4.6†	120.8±5.9†	45.7±2.4†	0.64±0.02†
NC	90.8±1.5*	148.7±6.1*	35.5±1.9*	0.66±0.01†

Abbreviations used and units: GPT, Serum Glutamate Pyruvate Transaminase as IU/L; GOT, Serum Glutamate Oxalate Transaminase as IU/L; Urea as mg/dl; Creatinine as mg/dl. Values are means ± SEM; *n* = 5. Differences between values with matching symbol notations within each bar are not statistically significant at 5% level of probability.

On the other hand, kidney is considered to be an important target organ for fluoride toxicity. We do observed significant changes in plasma urea and creatinine levels on exposure to F. However, treatment with Nanocurcumin did not revealed any significant prevention which needs further exploration.

## 3.6 Urinary 8-OHdG Levels (a Biomarker of DNA Damage)

Figure 5 represents the effect of nanocurcumin on arsenic and fluoride induced DNA damage in terms of 8-OHdG level in rats. Arsenic and fluoride significantly enhanced the level of 8-OHdG in the groups exposed with arsenic and fluoride individually and concomitantly although to different degrees. Nanocurcumin prevented DNA damage in As exposed group, however the effect was not observed when the two toxicants were administered concomitantly.

3.7 Arsenic and Fluoride Levels

A chelating effect of nanocurcumin was observed in blood as evidenced by significant depletion of arsenic and fluoride concentrations (Fig. 6).
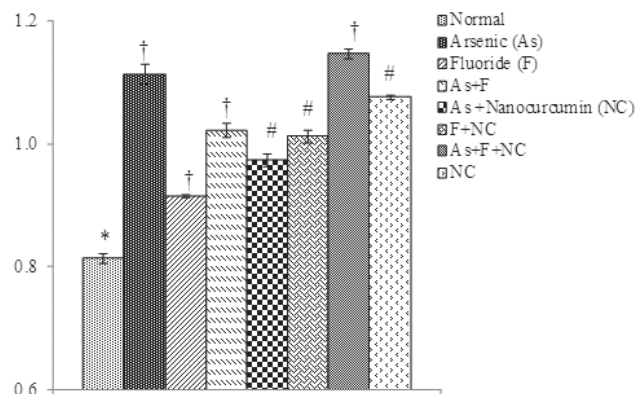
Figure 5. Effect of nanocurcumin supplementation on arsenic and fluoride induced DNA damage (8-OHdG) in rats. Values are mean ± SEM, *n* = 5. Values with the same symbol within each bar are not significantly different at the 5% level of probability.
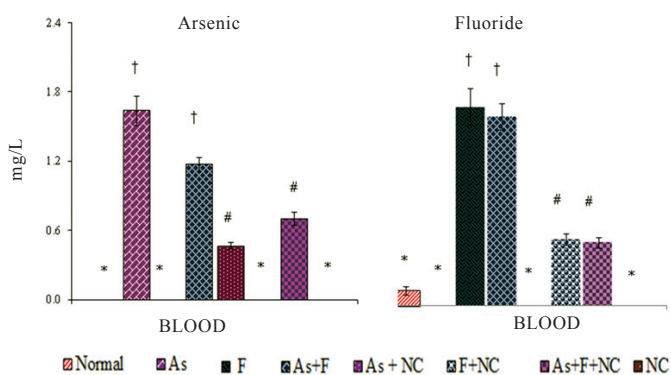
Figure 6. Effect of nanocurcumin supplementation on arsenic and fluoride concentration in rat blood. Values are mean ± SEM, *n* = 5. Values with the same symbol within each bar are not significantly different at the 5% level of probability.

4. DISCUSSION

The present study is in continuation of earlier report which established the efficacy of encapsulated curcumin nanoparticles in arsenic toxicity[16]. The results clearly demonstrated that curcumin encapsulated in chitosan nanoparticles retains its antioxidant and metal-chelating properties. This method of delivering curcumin to the cells overcomes its limitation of low bioavailability, thus providing a method to improve its clinical efficacy.

Elevated concentrations of naturally occurring arsenic (As) or fluoride (F) in drinking water is a worldwide problem and these two elements are recognised worldwide as the most serious inorganic contaminants in drinking water. Combined arsenic and fluoride poisoning through drinking water may cause either more severe or less pronounced toxicity. Contradictory results (synergistic or antagonistic) in different animal models suggest to the hypothesis that at low concentration fluoride ions are sufficient enough to react with arsenic however at high concentration the effects are predominantly of free fluoride ions compared to arsenic[11]. Fluoride may be able to ameliorate the toxic

effects of arsenic either through strong bond with arsenic or may be able to decrease its affinity for active cell components. Decreased toxicity of arsenic and fluoride co-exposure can be exposed on the basis of ionisation. Sodium fluoride is an ionic compound and gets completely ionised in aqueous solution.

Apart from affecting organs like liver and kidney it has been shown that inflammatory responses may play a crucial role in the mechanism of the cardiovascular toxicity of arsenic and fluoride[25]. Reports have also indicated that individuals exposed to As or F or As/F show a different pattern of gene expression compared to non-exposed subjects and thus have different consequences at cellular and genomic levels[26]. Chinoy and Shah have reported altered histology of cerebral hemisphere following combined arsenic-fluoride exposure in wherein the effects produced by arsenic were more prominent as compared to fluoride[27]. Decreased activity of antioxidant enzymes and increased lipid peroxidation is also reported following combined arsenic and fluoride exposure. Increased activity of SOD, catalase and GPx and increased thiol status has also been reported following combined exposure to these toxicants[27]. Genotoxic effects of the combined exposure to As and F were reported to be more pronounced as compared to their individual exposure[28-29].

Curcumin and its derivatives have shown the ability of being free-radical scavenger, interacting with oxidative cascade, quenching oxygen, chelating and diminishing oxidative properties of metal ions. It has been investigated in ameliorating heavy metal toxicity[30-33]. However, the major problem associated with the use of curcumin is its bioavailability. Recently, a lot of researches have focused on site specific targeted delivery of drugs using nanoparticles as carrier molecules. Efficacy of drugs can be enhanced by targeting the drugs through nanoparticles as carriers. These particles have easy accessibility in the body due to its smaller particle size and can be transported to different body sites through blood circulation.

Co-administration of nanocurcumin with arsenic and fluoride resulted in lowering of reactive oxygen species and restoration of blood glutathione level which were found to be altered in arsenic and fluoride intoxicated groups. Nanocurcumin also found to be effective in reversing δ-aminolevulinic acid dehydratase (ALAD) inhibition caused by arsenic exposure. This could be attributed to the availability of sulfhydryl group and antioxidant effects of curcumin. Several studies established the ability of curcumin to inhibit the generation of the superoxide radicals[34] by the increased production of GSH, which is a major non protein thiol in living organisms and plays a crucial role in co-coordinating the body's antioxidant defence process. In agreement to the above argument curcumin has been shown to reduce the induction in the levels of ROS and increase the activities of antioxidant enzymes and the levels of SH-groups in vivo, when co administered with arsenic. Thus, curcumin administration may reduce oxidative stress thereby being beneficial against toxic effects of arsenic and fluoride by reducing oxidative stress[35].

Co-administration of nanocurcumin was proved to be effective in the recovery of altered ROS and TBARS levels, followed by improved GSH level, and GPx and GST activities in rat liver and kidney both. It may be illustrious that curcumin counteracts the arsenic and fluoride induced tissue damage by quenching the generation of ROS. Due to the presence of polyphenolic structure and a β-diketone functional group curcumin has stronger antioxidant inhibitor properties than other flavonoids having single phenolic hydroxyl group. Our data proved that administration of arsenic and fluoride creates free radicals, which in turn alter the antioxidant systems, and necessarily lead to an increased lipid peroxidation. Nevertheless, co-supplementation of nanocurcumin along with these toxicants led to a significant decrease in lipid peroxidation and increased antioxidant activity. Our observation confirms the earlier finding which shows that administration of curcumin decreases the level of TBARS by improving the antioxidant status which is attributed to the longer circulation time of the nanoparticle in the blood which increases its bioavailability and hence the efficacy.

Earlier reports have demonstrated the efficacy of curcumin to chelate out heavy metals from the brain[30-31] as well as from liver and kidney[32] at a dose of ~100 mg/kg body weight curcumin which is quite high. Although, the ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs have also been shown at a lower dose of 15 mg/kg[35-36] but the data lacked any information on the metal content. The most promising result from our study shows that curcumin encapsulated chitosan nanoparticles at a dose of 15 mg/kg removes not only arsenic but also fluoride from blood which is due to the chelating potential of nanocurcumin. This can be attributed to the unique property of the chitosan nanoparticle which has longer circulation time in blood and helps in a sustained release of curcumin. Nano-encapsulation of curcumin may allow drug circulation and retention in the body thus not only reducing the dose but also maintaining its threshold bioavailability.

Thus in light of the present information, we conclude nanocurcumin successfully ameliorates the toxic effects of arsenic and fluoride in rat blood and tissues. The results also recorded the enhanced antioxidant and chelating potential of curcumin encapsulated chitosan nanoparticles at a much lower dose as a treatment regimen for both arsenic and fluoride toxicity. The results from our study have added a new dimension in the treatment of metal poisoning. We also hypothesise that a combination drug delivery therapy employing two nanosystems- a chelator and a strong anti-oxidant may produce synergistic effects in the treatment of arsenic and/or fluoride toxicity. We plan to investigate the same in our future studies to get into an insight into the mechanistic picture and provide answers to some of our unanswered experimental observations.

Conflict of Interest : None

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