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# CHARACTERIZATION AND SYNTHESIS OF ARYL ACRYLIC ACID SUBSTITUTED DERIVATIVES AND THEIR ABILITY FOR PREVENTION OF CADMIUM TOXICITY IN BIOLOGICAL SAMPLES

Chemistry

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## ABSTRACT

**Aim:** The objective of the present study was to synthesis & characterization of aryl acrylic acid substituted derivatives and investigate their ability to prevent cadmium toxicity in biological samples. **Method:** Purity of the substituted aryl acrylic acid derivatives was determining by high performance liquid chromatography (HPLC). For determination of purity, RP-HPLC method uses a HC-C18(2) analytical HPLC column, make Agilent Technologies (250 x 4.6mm). Mobile phase containing - Buffer (0.1%v/v H3PO4) and Acetonitrile (90: 10v/v), were used in the gradient elution with a flow rate of 1.0 mL/min and detection wavelength at 228 nm. Further molecular mass was determined by UHPLC with MS. 24 rats were divided into three groups of eight rats each. The control group received distilled water whereas group II received CdCl2 (1.5 mg/4 ml/body weight) through gastric gavage for 21 days. Group III to Group VI received CdCl2 and was treated with synthesized compounds for 21 days. α-Mercapto-β-acrylic acid derivatives were significant in reinstate normal blood profile and biochemical enzymatic levels of Cd-induced toxic animals. **Result:** Purity of substituted aryl acrylic acid derivatives was found more than 95% (Except compound C6) and C4 derivative was found most effective by restoring hemoglobin level to 10.98 g/dL and reduced Cd level in blood to 4.59 µg/mL as compared to toxic control group. **Conclusion:** The method was found to be adequate for the determination of purity of α-mercapto-β-aryl acrylic acid derivatives. Treatment with α-mercapto-β-aryl acrylic acid derivatives decreased the cadmium level in blood and tissues in all groups. 'α-Mercapto-β-acrylic acid derivatives were found effective in reducing CD toxicity without any adverse effect in rats.. We can conclude that the synthesized α-Mercapto-β-acrylic acid derivatives are effective against Cadmium toxicity.

## KEYWORDS

RP-HPLC, Purity, Cadmium toxicity, acrylic acid, Xanthine Oxidase and Total Thiol

## INTRODUCTION

Cadmium is a heavy metal posing severe risks to human health and living creatures. The exposure of cadmium leads to disturbance of respiratory system (shortness of breath, lung edema and destruction of mucous membranes), kidney damage, affecting reproductive system (low birth weight, spontaneous abortion, affecting production of progesterone and testosterone), bone damage, causing cancer etc. Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from batteries, electroplating, pigment, plastic, fertilizer industries, and cigarette smoke. It is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues[1]. Cd operates by various mechanisms of toxicity in particular species and under different experimental conditions[2]. Cd was found to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs, causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis[3]. In many countries, Cadmium toxicity has been reported that affects organs and causes death annually. Cadmium was shown to induce apoptosis in mouse liver[4].

Metals are the important class of toxic substance that is associated with day-to-day life of humans. The metals impose hazardous effect on humans' health and also affecting the environment [5]. They interfere with the metabolic processes of the body and disturbing biological system. The intensity of metallic toxicity depends upon the exposure rate, route, absorbed dose and duration of exposure [6]. The production of free radicals in the body due to metal intoxication is the very common cause of hazardous effect in the body [7]. The health issues associated with the intoxication of metals include neuronal damage, renal injuries, cardiovascular disorders, risk of diabetes and cancer[8]. Cadmium toxicity has been reported in many countries. It is a global health problem that affects organs and causes death annually[9].

Cadmium (Cd) is a naturally occurring heavy metal that cause severe injuries in humans and other living beings. It has long half-life up to 30 years in the body [10]. Cadmium produces genotoxicity by indirectly inducing oxidative stress in cells due to inhibition of antioxidant enzymes and depletion of antioxidant molecules. It has also been shown to inhibit DNA repair[11]. According to World Health of Organization, Cd exposure at low-level (acute toxicity) causes organ failure followed by lungs and gastrointestinal (GI) toxicity[12]. On the other hand, exposure at the high-level (chronic toxicity) leads to cancer and toxicities of organs such as cardiovascular, nervous, respiratory, urinary and reproductive systems. The very common cause involved in metal-induced toxicity is the production of free radicals as reactive oxygen species that leads to disruption of biochemical homeostasis at cellular level [13]. It induces tissue injury due to oxidative stress, inhibition of transport pathway particularly in proximal segment of the kidney and changes in DNA expression [14]. Cd level in the body can be measured in the samples of urine, blood, nail, hair and saliva. The toxicity of Cd to the patients required irrigation of GI tract, supportive care and detoxication with chelation therapy using new chelating agents [15]. The aim of this study was to investigate the protective effect of α-Mercapto-β-acrylic acid derivatives on hematological, biochemical and some enzymatic parameters in blood and tissues of cadmium exposed rats.

Chelation therapy has been used as a preferred therapy for minimizing the toxic effects of metals. These agents bind with toxic metal ions and form complex structures that can be easily excreted from the body and removing them from intracellular or extracellular spaces[16]. Chelation occurs between chelating agents and the resulting metal ion. Heavy metal toxicity can be effectively control with proper chelating agent. For chelation with Cd the conventional antidotes are available. So, researchers are looking for new compounds that have good low toxicity and having good chelation properties, For reducing Cd toxicity, Thiol chelating agents have been used as effective compounds [17]. α-Mercapto-β-acrylic acid derivatives are thiol chelators that is believed to act by inducing metallothionein (MT) biosynthesis and that is capable of binding with Cd [18]. Therefore, some α-Mercapto-β-acrylic acid derivatives were synthesized in present study as chelating agent to reduce Cd toxicity in rats. Reduction of metal toxicity was determined by observing the level of Cd in blood and tissues of rats.

## MATERIALS AND METHODS

### Chemical And Reagents

All biochemical used in the present study, Acrylic acid, rhodanine, cadmium chloride and trichloroacetic acid were procured from Sigma-Aldrich, USA. The other chemicals, reagents and solvents used in the study were of analytical grade.

### Design Of Experiment

### Reaction Schemes For Synthesis Of Substituted Aryl Acrylic Acid Derivatives:

**First Reaction Scheme:** A yellow suspended solution of the

Rhodanine adducts in 1N NaOH solution (27.8 g in 700 ml $H_2O$, 4.5 equiv.) was stirred at room temperature under an argon atmosphere. Mild heating and swirling of the reaction mixture (20 minutes) facilitated the stirring; a clear dark orange solution was obtained after 140 minutes at room temperature. The mixture continued stirring overnight for a total of 18 hours. The reaction was completed after overnight stirring as observed using TLC. The mixture was acidified carefully with 6N HCl at 0℃. Fine white particles dropped out of the solution initially then changed to a sticky brown gum. The impure product was used immediately for the next Benzothiophene ring closure reaction.

**Second Reaction Scheme:** (2Z)-3-(2-fluoro-5-methoxyphenyl)-2-mercapto-2-propenoic acid, 5-(2-fluoro-5-methoxyben-zylidene)-2-thioxo-1, 3-thiazolidin-4-one (8.00G, 9.7 mmol) was added in one portion to 25% w/v sodium hydroxide solution (40 mL). This was allowed stir at reflux for 1 h. The reaction was allowed to cool at room temperature and poured onto water (50 mL) after 1 h. It was washed with dichloromethane (50 mL), and the aqueous layer acidified to pH 2 with aqueous hydrochloric acid (2 N, 50 mL) to give a white suspension. Product was extracted with ether (2 × 60 mL), dried (MGS04) and solvent removed under vacuum to give a white solid. Purified this white solid material with dilute sodium hydroxide solution to get α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid.

**Third reaction scheme:** 5-(2-fluoro-5-methoxybenzylidene)-2-thioxo-1, 3-thiazolidin-4-one (8.00G, 9.7 mmol) was added in one portion to 25% w/v sodium hydroxide solution (40 mL). This was allowed stir at reflux for 1 h. The reaction was allowed to cool to room temperature and poured onto water (50 mL) after 1 h. It was washed with dichloromethane and the aqueous layer acidified to pH 2.0 with aqueous hydrochloric acid (2 N, 50 ML) to give a white suspension. The Product was extracted with ether (2 × 60 mL), dried (MGS04) and solvent removed vacuum to give a white solid mass, further purification done with sodium hydroxide of white solid to get α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid.

**Fourth reaction scheme:** A solution of 174.5 g of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl] methylene]-2-thioxo-4-thiazolidinone in 1250 ml of a 10% sodium hydroxide solution was heated on a steam bath for four hours. Decolorizing carbon was added and the mixture filtered through a high flow diatomaceous earth pad. The filtrate was chilled by adding ice and treated with 6N hydrochloric acid. The precipitated product was recovered by filtration, washed with water, and dried providing about 150 g of α-Mercapto-β-m-methoxy, (p-hydroxyphenyl) acrylic acid.

**Fifth reaction scheme:** Alkaline hydrolysis performed for 5-(aryl methylene) rhodanines to get the compound i.e. α-Mercapto-β-(o-nitro phenyl) acrylic acid

**Sixth reaction scheme:** Solution of the rhodanine was added to [4-(chloro-diphenyl-methyl)-phenoxy]-poly[styrene-co-divinylbenzene] containing 95% KOAc that was heated to 18 h with sodium hydroxide, potassium acetate in water & in acetic acid. Finally, get α-Mercapto-β-[ethyl-2- (amino)-4-thiazoleglyoxylate] acrylic acid.

## Characterization

Synthesized compounds were characterized by elemental analysis and melting points determination. Elemental analysis was performed by Elemental Analyzer (Make: Perkin Elmer, Model: 2400 Series II) and melting point were determined on Melting Point Apparatus (Make: Veego, Model- VMP-CM) and were uncorrected. Purity of new synthesized compounds were determined by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC).

## Experimental Section

In the present work, efforts have been made for the determination of purity of α-mercapto-β-aryl acrylic acids derivatives. Several trials have been made with respect to the mobile phase composition, columns, as well as UV detector's wavelength to develop a suitable and fast method for determination of purity and molecular mass of the synthesized compounds.

## Reagents And Chemicals

HPLC grade Acetonitrile (Make: Finar) and AR grade o-phosphoric acid were obtained from Merck, Darmstadt, Germany. Milli Q water used for analysis purpose.

## Equipment

High performance liquid chromatographic (HPLC) instrument used was Shimadzu make, model-LC-2010 CHT having Photo-Diode Array detector (PDA). Class VP Software 2.0 was used for data interpretation and acquisition.

## Chromatographic Conditions

Chromatographic column, HC-C18(2) make Agilent Technologies with dimensions (250 mm × 4.6 mm) was used as a stationary phase. Using mobile phase contained Buffer (0.1%v/v $H_3PO_4$) and Acetonitrile, was delivered at flow rate of 1.0 mL per minute. The eluent was detected at 228 nm at 25°C. The described HPLC method used for the quality and purity of the compounds. The developed Liquid Chromatographic method offers peak purity, symmetric peak shape and reasonable retention time for the compounds.

For determination of molecular mass of the derivatives, worked on ultra-high-performance liquid chromatography (UHPLC) system with mass detector and utilized the direct injection of 10 µL volumes. The separation was accomplished by using a HILIC column with a gradient elution program. Ammonium acetate solution (pH = 6.8) and acetonitrile were applied in the method as the mobile phase. The possible mass of acrylic acid was proposed in this work with the help of the product ion spectra of acrylic acid[19].

## Preparation of Sample Solution

Accurately weight & transfer about 25mg each of derivatives in separate 25mL volumetric flask (A class). Dilute with mobile phase and make up volume up to the mark with mobile phase. (Sample concentration about 1mg/ml). For UHPLC, Sample were prepared with acetonitrile without any further pretreatment. Finally, samples were filtered with 0.22 µm membrane filter[20].

## Animals for experiment

The animals were obtained from the animal house facility of Venus Medicine Research Centre, Baddi, H.P. The experiment was carried out after approval from the Institutional Animal Ethics Committee (IAEC). The study was performed on male wistar rats weighing 140±10 g, housed in polypropylene cages in an air-conditioned room with temperature maintained at 25±2 °C and 12 h alternating day and night cycles. The animals were allowed standard rat chow diet and sterile distilled water.

## Treatment Of Animals

For induction of toxicity, Cadmium chloride ($CdCl_2$) was given to the animals in the concentration of 1.5 mg/kg through an oral route. Loss in weight, decrease in hemoglobin, appetite loss and increase in body temperature showing signs of toxicity in the animal. Animals were categorized into eight groups each containing five animals; Group – I was negative control group containing normal saline, Group – II was toxic control group containing $CdCl_2$ 1.5 mg/kg, Group III to VIII containing synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives. All treated groups were administered with 155 mg/kg of synthesized compounds orally. All the animals were treated daily for 21 days after producing toxicity.

## Plasma preparation from blood samples

Sample preparation method combining solid-phase extraction (SPE) and liquid-liquid extraction (LLE) was developed to be used in Effect-Directed Analysis (EDA) of blood plasma, until now such a method was not available. Blood (about 1.5 ml) was centrifuged at 6000 rpm for 15 min, the supernatant was removed and taken into other polypropylene tube. It was stored at 2-8℃ for the measurement of enzymatic and oxidative stress parameters.

## Collection Of Tissues And Preparation Of Homogenate

After last treatment for 24 hours, all animals were decapitated. About 2.5 ml blood was collected in EDTA containing vials. Tissues such as liver, kidney and brain were collected in chilled phosphate buffer saline and washed three to four times with chilled phosphate buffer saline (PBS) and homogenates were prepared for measurement of biochemical parameters. Tissue homogenates were prepared by taking tissues in chilled phosphate buffer-NaCl solution containing 0.15 mol/L NaCl in 0.05 mol/L $Na_2HPO_4$ - $NaH_2PO_4$ buffer (pH 7.2). The solution was left for at least 1 h at 2–8℃ before measurement of biochemical parameters[21].

**Determination Of CD Concentration In Blood And Tissues**

Take 0.5 ml samples each of blood, liver, kidney and brain were mixed with 4.5 ml of acidic glycerol (1% $HNO_3$ and 5% glycerol mixture) and the final volume of 10.0 ml was obtained with distilled water. Cadmium was measured by using a flame atomic absorption spectrophotometer (Analytikjena Contra A300, Germany) with hollow-cathode lamp at wave length 228.8 nm. The direct absorption of the solution was determined by the atomic absorption spectrophotometer and suitable standard curves of each metal were prepared by using 10 to 100 µg/ ml [22].

**Estimation Of Xanthine Oxidase And Total Thiol**

Key enzyme {like: Xanthine oxidase (XO)} is involved in variety of diseases related to vital organs of the body. In the present study, the substituted derivatives of aryl acrylic acids were evaluated for xanthine oxidase inhibitory and antioxidant activities. XO is an enzymatic source for generation of reactive oxygen species (ROS) and increase reactive oxygen species (ROS) may lead to cellular damages and progression of several diseases[23].

Thiols are the organic compounds that contain a sulphydryl group. Among all the antioxidants that are available in the body, thiols constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species. Thiol status in the body can be assessed easily by determining the serum levels of thiols. Decreased levels of thiols has been noted in various medical disorders including chronic renal failure and other disorders related to kidney, cardiovascular disorders, stroke and other neurological disorders, diabetes mellitus, alcoholic cirrhosis and various other disorders. Therapy using thiols has been under investigation for certain disorders [24].

**Statistical Analysis**

All values were presented as Mean ± SD(n=6). Statistics were performed using a one-way ANOVA followed by Newman-Keuls comparison test. Results were statistically different at ***p<0.001, **p<0.01, and *p<0.05 using Graphpad Prism software version 5.0 in comparison to toxic control group.

**RESULT**

**Synthesis And Characterization Of Some Substituted Aryl Acrylic Acid Derivatives**

IUPAC name of synthesized α-mercapto-β-aryl acrylic acid derivatives are: C1, α-Mercapto-β-thienyl acrylic acid; C2, α-Mercapto-β-(p-methoxyphenyl) acrylic acid; C3, α-Mercapto-β-(p-dimethyl aminophenyl) acrylic acid; C4, α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid; C5, α-Mercapto-β-(o-nitrophenyl) acrylic acid; and C6, α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] acrylic acid. Characterization data of all 06 substituted aryl acrylic acid derivatives as shown in Table-1 and Table-2. UHPLC method provided good recovery from 101.6% to 103.8%, good precision with intra-day relative standard deviations in a range of 1.8% and 4.8%. Limit of quantification for the method was 40 µg/L.

**Table – 1: Data For Characterization Of Substituted Aryl Acrylic Acid Derivatives**

| Compounds | IUPAC name | Molecular Formula | Yield | M.P. (°C) |
|---|---|---|---|---|
| C1 | α-Mercapto-β-thienyl acrylic acid | ($CH_6O_2S_2$) | 81% | ~135 |
| C2 | α-Mercapto-β-(p- methoxyphenyl) acrylic acid | ($C_{10}H_{10}O_3S$) | 73% | >300 |
| C3 | α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid | ($C_{11}H_{13}O_2SN$) | 78% | ~170 |
| C4 | α-Mercapto-β-m- methoxy, (p-hydroxy phenyl) acrylic acid | ($C_{10}H_{10}O_4S$) | 82% | ~210 |
| C5 | α-Mercapto-β-(o-nitrophenyl) acrylic acid | ($C_9H_7O_4SN$) | 72% | ~260 |
| C6 | α-Mercapto-β-[ethyl-2- (amino)-4-thiazole glyoxylate] acrylic acid | ($C_{13}H_{15}NO_5S$) | 68% | >260 |

**Table – 2: Spectral & Elemental Analysis Result**

| Compounds | Retention Time (By HPLC) | Purity | Elemental Analysis (%) | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| C1 | 2.8 min. | 97.6% | Calculated = 45.15<br>Found = 45.09 | 3.20<br>3.03 | -<br>- |
| C2 | 4.2 min. | 98.2% | Calculated = 57.14<br>Found = 57.23 | 4.71<br>4.62 | -<br>- |
| C3 | 5.2 min. | 96.8% | Calculated = 59.22<br>Found = 59.41 | 5.40<br>4.95 | 6.32<br>6.71 |
| C4 | 6.2min. | 99.8% | Calculated = 53.10<br>Found = 53.19 | 4.42<br>4.39 | -<br>- |
| C5 | 7.8 min. | 95.4% | Calculated = 48.00<br>Found = 47.56 | 3.11<br>3.29 | 6.22<br>6.53 |
| C6 | 8.8 min. | 92.8% | Calculated = 39.73<br>Found = 39.91 | 3.31<br>3.46 | 9.29<br>9.80 |

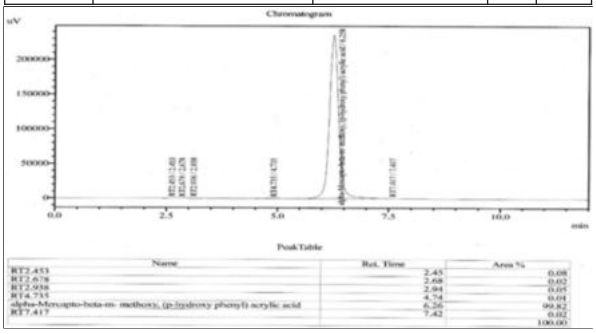



**Figure 1:** Representative HPLC chromatogram of Compound C4 [i.e. α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid]

**Blood Profile Results Of Animals And Effect Of Synthesized Compounds**

Hematological parameters results exhibited that synthesized compounds were effective in reducing cadmium toxicity after 21 days in cadmium exposed animals as showed in Table – 3.

**Table – 3 Hematological Parameters Result In Cadmium Exposed Animals**

| Groups | Hb (g/dL) | RBC ($10^6$/ L) | HCT (%) | WBC ($10^3$/ L) |
|---|---|---|---|---|
| CG | 11.40±0.66*** | 8.88±0.21*** | 40.11±1.04*** | 5.91±0.59*** |
| TG | 8.37±1.11 | 7.66±0.58 | 31.28±2.00 | 12.01±1.09 |
| C1 | 10.14±0.84*** | 7.16±0.58$^{ns}$ | 39.02±1.06* | 10.02±1.07* |
| C2 | 9.88±0.72* | 7.33±0.50$^{ns}$ | 37.04±0.44* | 8.25±0.94** |
| C3 | 9.87±0.49* | 7.55±0.51$^{ns}$ | 39.93±1.17* | 9.09±1.09* |
| C4 | 10.98±0.93*** | 8.33±0.24*** | 40.02±0.80*** | 6.03±0.44*** |
| C5 | 10.40±0.80** | 7.69±0.38** | 37.51±2.48$^{ns}$ | 9.52±1.92$^{ns}$ |
| C6 | 9.66±0.89$^{ns}$ | 7.55±0.29* | 36.11±1.97$^{ns}$ | 9.69±1.60$^{ns}$ |

Values are represented Mean ± SD (n=6), significantly different at ***P<0.001, **p<0.01, *p<0.05 and not significant ns>0.05 in comparison to toxic control group. CG : Control group, TG: Treated group, Hb: Hemoglobin, RBC: Red blood cells, HCT: Hematocrit, WBC: White blood cells,

In which compound C4 [α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid] showed significant protecting activity. Hemoglobin content of C4 compound was 10.98 g/dL, which was significant (***P<0.001) in comparison to toxic control group (8.37 g/dL). The other test drugs treated groups were also effective in against Cd toxicity but in lesser extract as compound C4. Likewise, other hematological parameters including RBC and HCT were significantly (***P<0.001) raised with the treatment of C4 compound i.e. 8.33 and 40.02, respectively that was highest among different treated groups. In contrast, the WBC count of the toxicity induced group was raised (12.01) in comparison to the untreated control group (5.87). C4 compound was significantly (***P<0.001) reduced WBC count (6.03) in blood samples of Cd exposed animals.

**Cadmium Level In Blood And Tissues After Treatment & Their Effect**

Cd level decreased in blood and tissues in all groups after treatment α-mercapto-β-aryl acrylic acid derivatives. Table – 4 shows the cadmium (Cd) level in the blood and tissues of animals. Reduction in Cd level was significant (***P<0.001) with the compound C4. The amount of Cd in blood was reduced to 4.59 µg/mL in C4 treated group, which was significant in comparison to the toxic control group (11.96 µg/mL). Similarly, C4 compound [α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid] was significantly (***P<0.001) reduced cadmium level in liver & kidney and reached to 8.60, 7.51 µg/g tissue,

respectively in comparison to toxic control group animals i.e. 16.91 (liver) and 14.77 (kidney). However, reduction in tissue cadmium level was also seen with other α-mercapto-β-aryl acrylic acid derivative compounds.

**Table – 4: Cadmium Level In Blood, Liver And Kidney**

| Groups | Blood ( g/mL) | Liver ( g/g tissue) | Kidney ( g/g tissue) |
|---|---|---|---|
| CG | 0.15±0.01*** | 0.62±0.12*** | 0.19±0.03*** |
| TG | 11.96±0.80 | 16.91±1.24 | 14.77±1.25 |
| C1 | 11.61±1.11 ns | 15.09±1.44 ns | 12.43±1.27 ns |
| C2 | 10.91±1.15* | 14.60±1.87* | 12.10±1.05 ns |
| C3 | 10.47±1.09** | 14.85±0.67* | 11.26±1.69* |
| C4 | 4.59±0.53*** | 8.60±0.62*** | 7.51±1.09*** |
| C5 | 11.60±0.90 ns | 14.19±1.11** | 11.89±2.66 ns |
| C6 | 10.97±0.55* | 15.81±0.81 ns | 11.70±2.42* |

Values are represented Mean ± SD (n=6), significantly different at ***P<0.001, **p<0.01, *p<0.05 and not significant ns>0.05 in comparison to toxic control group.

**Synthesized Compounds Effect On Xanthine Oxidase (XO)**

Xanthine oxidase (XO) and Glutathione-S transferase (GST) activities were significantly altered in the plasma and tissues of the $CdCl_2$ induced group as compared with the control group. The XO activity was reduced (Table – 5) in plasma and tissues of all treated groups as compared to the $CdCl_2$ induced group after 21 days treatment.

**Table – 5 Effect Of Synthesized Compounds On Xanthine Oxidase (XO) Level**

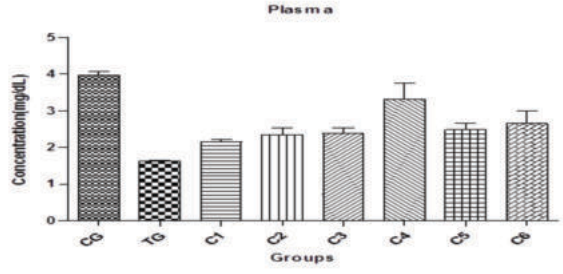
| Groups | Plasma | Liver | Kidney |
|---|---|---|---|
| CG | 135.26±4.05*** | 44.07±3.01*** | 49.22±2.29*** |
| TG | 301.64±8.0 | 95.05±2.88 | 67.13±2.60 |
| C1 | 289.26±5.33 ns | 86.14±1.81 ns | 60.71±4.77 ns |
| C2 | 285.70±8.45 ns | 83.66±3.29* | 58.22±1.09** |
| C3 | 280.83±11.00 ns | 80.96±5.01** | 60.38±7.55 ns |
| C4 | 262.53±22.39*** | 58.15±4.58*** | 50.96±1.94*** |
| C5 | 285.81±6.24 ns | 83.79±8.64* | 62.05±5.77 ns |
| C6 | 273.31±16.69*** | 8446±10.66 ns | 63.72±5.09 ns2 |

Values are represented Mean ± SD (n=6), significantly different at ***P<0.001, **p<0.01, *p<0.05 and not significant ns>0.05 in comparison to toxic control group.

XO level was expressed in the plasma (mg/dL) whereas in the tissues (μmol/mg protein).

**Total Thiol Content**

Total thiol level was significantly lowered in plasma, liver and renal tissue of the cadmium exposed group in comparison to control group. Total thiol level was significantly elevated in plasma and tissues of the treated group after treatment with synthesized drugs for 21 days in comparison to toxic group. Rise in the level of total thiol was more significant with the C4 synthesized compound. The level of total thiol was 3.29±0.96 mg/dL in plasma, 4.33±0.69 μmol/mg protein in liver and 69.13±1.05 μmol/mg protein in kidney, which was comparable with toxic control group i.e. 1.67, 2.74 and 57.22 in plasma, liver and kidney, respectively. However, total thiol level of α-mercapto-β-aryl acrylic acid derivatives was 2.16, 3.29 and 63.91 for C1, α-Mercapto-β-thienyl acrylic acid; 2.33, 3.41 and 60.05 for C2, α-Mercapto-β-(p-methoxyphenyl) acrylic acid; 2.39, 2.81 and 58.66 for C3, α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid; 2.50, 2.99 and 7.75 for C5, α-Mercapto-β-(o-nitrophenyl) acrylic acid; and 2.68, 3.30 and 52.27 for C6, α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] acrylic acid in plasma, liver and kidney, respectively as shown in the figure-2.



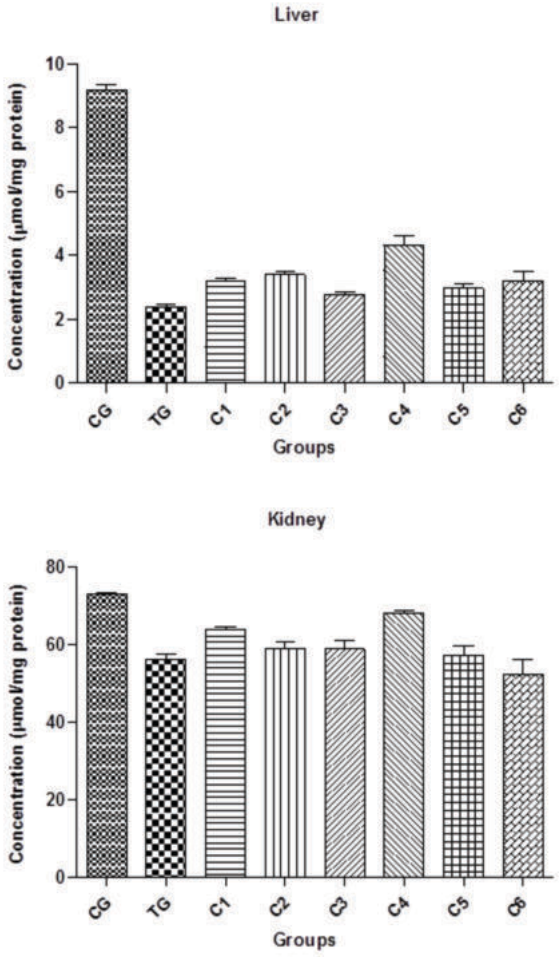




**Figure -2:** Total Thiol In Cadmium Exposed Animals After 21 Days Of Treatment

**DISCUSSION**

For more effective treatment of cadmium poisoning, some mercapto acrylic acid derivatives have been synthesized according to the scheme and their ability to remove cadmium from the sub-cellular fractions of liver and kidney was tested in cadmium pre-exposed rats. However, the substitution on the aryl ring at β-position in α-mercapto-β-aryl acrylic acids has any influence on their cadmium mobilizing ability was also investigated. In the present study, six different α-Mercapto-β-acrylic acid derivatives were prepared using stearyl methylene rhodanine to reduce the cadmium (Cd) toxicity by complexation with it. Results exhibited that compounds C4 (α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid) was most effective for complexation with Cd. The toxicities in blood and tissues (i.e. liver and kidney) were reduced after treatment with α-Mercapto-β-acrylic acid compounds. Treatment with α-mercapto-β-aryl acrylic acid derivatives decreased the cadmium level in blood and tissues in all groups. Xanthine oxidase (XO) activities were significantly altered in the plasma and tissues of the $CdCl_2$ induced group as compared with the control group. The antioxidant activity was more significant with the C4 group.

Total thiol level was significantly elevated in plasma and tissues of the treated group after treatment with synthesized drugs for 21 days in comparison to the toxic group. This rise in the level of total thiol was more significant with the C4 synthesized compound among other synthesized compounds.

Xanthine oxidase (XO) activities were significantly altered in the plasma and tissues of the $CdCl_2$ induced group as compared with the control group. The antioxidant activity was more significant with the C4 group. It increased XO activity in plasma (262.33 mg/dL), in liver (58.15 μmol/g tissue) and in kidney (50.96 μmol/g tissue), which was comparable with toxic control group i.e. 301.64, 95.05 and 67.13 in plasma, liver and kidney, respectively.

## CONCLUSION

Cadmium level decreased after treatment with α-mercapto-β-aryl acrylic acid derivatives in blood and tissues in all groups. Total thiol level was significantly lowered in plasma, liver and renal tissue of the cadmium exposed group in comparison to the control group. Xanthine oxidase (XO) were significantly altered in the plasma and tissues of the $CdCl_2$ induced group as compared with the control group. The antioxidant activity was more significant with the C4 group. So, α-Mercapto-β-acrylic acid derivatives were found effective in reducing Cd toxicity without any adverse effect in rats. In addition to that, it was able to reduce the level of Cd in both blood and tissues and restored normal hematological and biochemical profile of animals. We can conclude that the developed HPLC method with PDA detector offers a simple method for purity estimation of aryl acrylic acid derivatives and the synthesized α-Mercapto-β-acrylic acid derivatives are effective against Cadmium toxicity.

## Declaration

The authors declared no conflict of interest.

## REFERENCES


1. Stohs & Bagchi, (1995 Feb): Oxidative mechanisms in the toxicity of metal ions: Free Radic Biol Med): 18(2); 321-326
2. Casalino E, Calzaretti G, Sblano C, Landriscina C. (2002a): Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicol;179:37–50
3. Michael PW, Bhalchandra AD. (1999-20): Cadmium-induced inhibition of the growth and metastasis of human lung carcinoma xenografts: role of apoptosis. Carcinogen :20:65–70
4. Engwa GA, Ferdinand PU, Nwalo FN, Unachukwu MN (2019): Mechanism and health effects of heavy metal toxicity in humans. Poisoning in the Modern World - New Tricks for an Old Dog? Intech Open Publication:1-23
5. Ali H, Khan E, Ilahi I. (2019): Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Yang Y, editor. J Chem.
6. Jan AT, Azam M, Siddiqui K, Ali A, Choi I, Haq QMR (2015): Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. Int J Mol Sci. ;16(12):29592–630.
7. Rehman K, Fatima F, Waheed I, Akash MSH. (2018): Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem: 119(1):157-184.
8. Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia AA (2017); Cadmium toxicity and treatment: An update. Caspian J Intern Med; 8(3):135-145
9. Berglund M, Larsson K, Grandér M, Casteleyn L, et al., (2015): Exposure determinants of cadmium in European mothers and their children. Environ Res: 141:69-76.
10. Hartwig, A., Asmuss, M., Blessing, H. Food Chem. Toxicol. 2002;40:1179–1184.
11. Air Quality Guidelines - Second Edition, WHO Regional Office for Europe, Copenhagen, Denmark, 2000
12. WHO. Cadmium. Environmental Health Criteria. WHO, Geneva, (1992) , p.280.
13. Fisher RM, Gupta V. (2020): Heavy Metals. In: StatPearls. Treasure Island (FL): StatPearls Publishing;
14. Bernhoft RA. (2013): Cadmium Toxicity and Treatment. Grant H, Hansen DK, editors. Sci World J:394652.
15. Ferrero ME. (2016): Rationale for the Successful Management of EDTA Chelation Therapy in Human Burden by Toxic Metals. Huang X, editor. Biomed Res Int: 8274504.
16. Swaran J.S. Flora and Vidhu Pachauri. (2010 Jul): Chelation in Metal Intoxication. International J Environ Res Public Health.; 7(7): 2745–2788
17. Bjørklund G, Crisponi G, Nurchi VM, Cappai R, Buha Djordjevic A, Aaseth J. (2019): A Review on Coordination Properties of Thiol-Containing Chelating Agents Towards Mercury, Cadmium, and Lead. Molecules;24(18):3247.
18. Tandon SK, Prasad S. (2000): Effect of thiamine on the cadmium-chelating capacity of thiol compounds. Hum Exp Toxicol: 19(9):523-8.
19. .Innocenzo Casella, Marianna Pierri, Michela Contursi (March2006) Determination of acrylamide and acrylic acid by isocratic liquid chromatography with pulsed electrochemical detection, Journal of Chromatography A 1107(1-2):198-203
20. Hao Zhang, Juam Wei, Zongli Hau, (2019), UPLC-MS/MS method for the determination of acrylic acid in tap water, Journal of Liquid Chromatography & Related Technologies, Volume 43, 2020 - Issue 5-6
21. Dwivedi VK, Arya A, Gupta H, Bhatnagar A, Kumar P, Chaudhary M. (2011): Chelating ability of sulbactomax drug in arsenic intoxication: African Journal of Biochemistry Research: 5(10), 307-314
22. S U Khan, R O Cloutier, M Hidiroglou (1979): Atomic absorption spectroscopic determination of molybdenum in plant tissue and blood plasma: Assoc Off Anal Chem : 62(5):1062-4
23. Hui Ouyang, Kun Hou, Wanxi Peng, Zhenling Liu, Heping Deng, (2018) : Antioxidant and xanthine oxidase inhibitory activities of totalpolyphenols from onion, Saudi Journal of Biological Sciences: 1509-1513
24. R Rossi, D Giustarini, A Milzani,and I Dalle-Donne : (2009): Cysteinylation and homocysteinylation of plasma protein thiols during ageing of healthy human beings: 13(9b): 3131–3140