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RESEARCH ARTICLE

PROTECTIVE ROLE OF α-MERCAPTO- β-ACRYLIC ACID SUBSTITUTED DERIVATIVES ON OXIDATIVE STRESS, LIPID PEROXIDATION, ANTIOXIDANT ENZYMATIC ACTIVITIES IN CADMIUM TOXICITY INDUCED RAT MODEL

Anuj Bhatnagar, Sarika Arora and Raj Kumar Singh
Department of Chemistry, IFTM University, Moradabad (UP)

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Abstract

Objective: Synthesize some new α-Mercapto-β-acrylic acidsubstituted derivatives, characterize them and investigate their protective role on oxidative stress, lipid peroxidation, antioxidant enzymatic activities and Cd levels in the blood and tissues of cadmium exposed rats.

Method: Twenty-four male 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. The lipid peroxidation level, oxidative stress and enzymatic parameters were measured in plasma and tissues (brain, liver and kidney) of all groupsα-Mercapto-β-acrylic acidderivatives were significant in reinstate normal blood profile and biochemical enzymatic levels of Cd-induced toxic animals. Result: C4 derivative was most effective by restoring hemoglobin level to 11.05 g/dL and reduced Cd level in blood to 4.64 µg/mL as compared to toxic control group. Xanthine oxidase (XO) and Glutathione-S transferase (GST) activities were significantly altered in the plasma and tissues of the CdCl2 induced group as compared with the control group. Total thiol level was significantly ($p<0.001$) decreased in α-Mercapto-β-acrylic acidderivatives treated group as compared to cadmium exposed group. Reduction in the MDA level and MPO level was more significant with C4 synthesized compound among other synthesized compounds.

Conclusion: Treatment with α-Mercapto-β-acrylic acidderivatives decreased the cadmium level in blood and tissues in all groups. without any major side effect, α-Mercapto-β-acrylic acidderivatives were found effective in reducing Cd toxicity in rats. We can conclude that the synthesized α-Mercapto-β-acrylic acidderivatives are effective against Cadmium toxicity.

Introduction:-

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

Corresponding Author:- Anuj Bhatnagar
Address: Department of Chemistry, IFTM University, Moradabad (UP)

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]. A number of cadmium-induced effects include deterioration of cell to cell adhesion, DNA-related processes cell signaling and energy metabolism, implying that this metal acts on different molecular targets in human organs. 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 upto 30 years in the body [10]. A number of cadmium-induced effects include deterioration of cell to cell adhesion, DNA-related processes cell signaling and energy metabolism, implying that this metal acts on different molecular targets in human organs. According to World Health of Organization, Cd exposure at low-level (acute toxicity) causes organ failure followed by lungs and gastrointestinal (GI) toxicity[11]. 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 [12]. It induces tissue injury due to oxidative stress, inhibition of transport pathway particularly in proximal segment of the kidney and changes in DNA expression [13]. 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 [14].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[15]. Chelation occurs between chelating agents and the resulting metal ion. With proper chelating agent heavy metal toxicity can be efficiently control. 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 [16]. 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[17]. Therefore, six 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.

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.

Design of Experiment

Six derivatives of acrylic acid were prepared in step wise reaction scheme by the alkaline of 5- (aryl methylene) rhodanines, obtained from the condensation of substituted aldehydes and rhodanine. Male rats categorized into eight groups each containing five animals:

1. Group I: Control normal saline treated group
2. Group II: $CdCl_2$ induced group (1.5mg /4ml /kg body weight)
3. Group III: $CdCl_2$ + Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-thienyl acrylic acid
4. Group IV: $CdCl_2$ + Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-(p-methoxy phenyl) acrylic acid
5. Group V: $CdCl_2$ + Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid
6. Group VI: $CdCl_2$ + Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-m-methoxy (p-hydroxyphenyl) acrylic acid
7. Group VII: $CdCl_2$ + Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-(o-nitro phenyl) acrylic acid
8. Group VIII: $CdCl_2$ +Synthesized compounds of α-mercapto-β-aryl acrylic acid derivatives i.e. α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] acrylic acid

All treated groups were administered with 155 mg/kg of synthesized compounds orally and were treated daily for 21 days after producing toxicity.Acute oral toxicity study of synthesized derivatives was performed according to OECD guideline 423. The animals were given different doses of compounds i.e. 50, 150, 300, 500, 1000 and 2000 mg/kg once and observed daily for 14 days regarding change in color eyes and behavioral change, and change in feeding and water, if any.

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. Weight loss, decreased hemoglobin, loss of appetite, and high 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 i.e. **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. 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

A 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. It can be used for extraction of a broad range of thyroid hormone (TH)-disruptors from plasma with high recoveries. Blood (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 homogenate preparation

The animals of all groups were decapitated for 24 hours after last treatment and then 2.5 ml blood was collected in EDTA containing vials. The tissues such as liver, kidney and brain were collected in chilled phosphate buffer saline and washed three times with chilled phosphate buffer saline (PBS) and homogenates were prepared for measurement of biochemical and enzymatic parameters. Tissue homogenates were prepared by taking tissues such as liver, kidney and brain 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 enzymatic and biochemical parameters[18].

Metal determination in blood and tissues

For assessment of cadmium (Cd) concentration in blood and tissues, 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 [1920].

Measurement ofXanthine Oxidase Activity

Xanthine oxidase (XO) is the key enzyme involved in variety of diseases related to vital organs of the body. In this study, the 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) more specifically for the abundant production of hydrogen peroxide and superoxide radicals. Increase ROS may lead to cellular damages and progression of several diseases[21]. Natural compounds have been used for the treatment of diseases as the prime source for the development of new drugs. However, poor biopharmaceutical properties and toxicity of herbal drugs always present challenge to improve their effectiveness by structural modification, which has been demonstrated by the synthetic or derived drugs.

Estimation of total thiol content

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. Total thiols composed of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins. Among the thiols that are bound to proteins, albumin makes the major portion of the protein bound thiols, which binds to sufhydryl group at its cysteine-34 portion. Apart from their role in defense against free radicals, thiols share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level. The 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[22.]

Oxidative stress parameters

Oxidative stress is an indication of imbalance between oxidants and respective defense systems of an organism. Oxidants mostly encompass reactive oxygen species (ROS), this oxidative radical in many cases able to propagate the reaction leading to extensive damage. The increased oxidative stress may lead to increased lipid peroxidation. The assessment of lipid peroxidation is usually performed by analyzing secondary oxidation products such as malondialdehyde (MDA)[23].

Estimation of Lipid peroxidation level

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malondialdehyde(MDA) formed, essentially according to method of[24]. It was determined by thiobarbituric reaction.

Estimation of Myeloperoxidase level (MPO)

Myeloperoxidase is an enzyme released by activated neutrophils and acts as a proinflammatory mediator. It is the most abundant protein found in neutrophils (also found in monocytes) represents inflammatory pathologies. It has reported that MPO is an excellent biomarker for human cardiovascular risk. Its ability to catalyze reaction between chloride and hydrogen peroxide (H_2O_2) to form hypochlorous acid is unique among mammalian enzymes and is considered to be the dominant function of MPO in vivo[25].

Hypochlorous acid is a powerful antimicrobial agent, and extremely reactive with biological molecules causing much of the damage mediated by neutrophils in inflammatory diseases. MPO also exhibits peroxidase activity that catalyzes oxidation of a number of substrates by (H_2O_2). This activity has been widely used to assess the amount of MPO. Unfortunately, its specificity is very poor for unpurified biological samples due to the presence of other peroxidases. However, peroxidases generally do not produce hypochlorous acid; the only exception is eosinophil that produces hypochlorous acid at pH levels below 5. The chlorination activity of MPO has a pH optimum of near neutral pH[26]. Therefore, assay conditions can be set so to provide for MPO enzyme specificity.

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

Some α-mercapto-β-aryl acrylic acid derivatives were synthesized and their IUPAC name 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 as shown in Table – 1.

Table 1:- Spectral data of thesynthesized aryl acrylic acid derivatives.

Compound (Molecular Formula)	Reacting Aldehyde	Melting Point (°C)	Yield (%)	IR (cm-1)	Elemental Analysis (%)(Cal./Found)			Purity (By HPLC)
					C	H	N	
C1 ($CH_6O_2S_2$)	2-Thenaldehyde	136-138	80	3.21 2.96	45.16 45.13	3.23 2.98	- -	97.62%
C2 ($C_{10}H_{10}O_3S$)	p-Methoxy-benzaldehyde	>300	74	4.76 3.57	57.14 57.19	4.76 3.94	- -	98.27%
C3 ($C_{11}H_{13}O_2SN$)	p-imethylamino-benzaldehyde	169-171	77	5.84 4.89	59.19 59.40	5.82 4.89	6.81 6.72	96.84%
C4 ($C_{10}H_{10}O_4S$)	m-methoxy, p-hydroxy-benzaldehyde	211-213	84	4.40 4.03	53.14 53.18	4.43 4.11	- -	99.84%
C5 ($C_9H_7O_4SN$)	o-nitro-benzaldehyde	>255	72	3.13 3.28	47.98 47.45	3.11 3.26	6.23 6.48	95.44%
C6 ($C_{13}H_{15}NO_5S$)	Ethyl-2-(formylamino)-4-thiazole glyoxylate	266	68	3.31 3.48	39.71 39.83	3.30 3.47	9.28 9.79	92.84%

Results and effect of synthesized compounds on blood profile of animals

Results of hematological parameters exhibited that synthesized compounds were effective in reducing cadmium toxicity after 21 days in cadmium exposed animals as showed in Table – 2.

Table 2:- Hematological parameters result and effect in cadmium exposed group.

Groups	**Hb (g/dL)**	**RBC (10^6/µL)**	**HCT (%)**	**WBC (10^3/µL)**
CG	11.41±0.61***	8.92±0.24***	40.02±1.09***	5.84±0.61***
TG	8.42±1.13	7.66±0.58	31.28±2.00	11.97±1.08
C1	10.09±0.84**1	7.11±0.57 ns	38.42±1.02*	9.91±1.04*
C2	9.88±0.72*	7.33±0.50 ns	37.04±0.44*	8.25±0.94**
C3	9.87±0.49*	7.50±0.51 ns	39.30±1.12*	9.06±1.11*
C4	11.05±0.90***	8.21±0.24***	39.72±0.78***	5.95±0.41***
C5	10.32±0.80**	7.69±0.38**	37.51±2.48 ns	9.52±1.92 ns
C6	9.60±0.87 ns	7.51±0.29*	36.11±1.95 ns	9.66±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.

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 11.05 g/dL, which was significant (***P<0.001) in comparison to toxic control group (8.42 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. 8.21 and 39.72, respectively that was highest among different treated groups. In contrast, the WBC count of the toxicity induced group was raised (11.97) in comparison to the untreated control group (5.84). C4 compound was significantly (***P<0.001) reduced WBC count (5.95) in blood samples of Cd exposed animals.

Effect of synthesized compounds on cadmium level in blood and tissuesafter treatment

Cd level decreased in blood and tissues in all groups after treatment α-mercapto-β-aryl acrylic acid derivatives. Table – 3 shows the level of cadmium (Cd) 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 4.64µg/mL in C4 treated group, which was significant in comparison to the toxic control group (12.68µg/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 brain and reached to 8.63, 7.47 and 2.59µg/g tissue, respectively in comparison to toxic control group animals i.e. 16.85 (liver), 14.71 (kidney) and 4.28 (brain). However, reduction in tissue cadmium level was also seen with other α-mercapto-β-aryl acrylic acid derivative compounds.

Table 3:- Effect of synthesized compounds on cadmium level in blood and tissues after 21 days of treatment.

Groups	Blood (µg/mL)	Liver (µg/g tissue)	Kidney (µg/g tissue)	Brain (µg/g tissue)
CG	0.13±0.01***	0.60±0.18***	0.17±0.03***	0***
TG	12.68±0.85	16.85±1.22	14.71±1.22	4.28±0.65
C1	11.59±1.10 ns	15.02±1.43 ns	12.43±1.27 ns	3.84 ±0.67 ns
C2	10.91±1.15*	14.60±1.87*	12.10±1.05 ns	3.10±0.37**
C3	10.47±1.09**	14.85±0.67*	11.26±1.69*	3.53±0.48 ns
C4	4.64±0.53***	8.63±0.59***	7.47±1.06***	2.59±0.05***
C5	11.55±0.91 ns	14.17±1.13**	11.93±2.63 ns	3.44±0.74 ns
C6	10.92±0.53*	15.77±0.83 ns	11.72±2.39*	3.25±0.49*

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 compoundseffect on Xanthine oxidase (XO) and Glutathione-S transferase (GST)

Effect of 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 – 4) along with increased in GST activity (Table – 5) in plasma and tissues of all treated groups as compared to the $CdCl_2$ induced group after 21 days treatment. The antioxidant activity was more significant with C4 group.

Table 4:- Effect of synthesized compounds on xanthine oxidase (XO) level in cadmium exposed animals after 21 days of treatment.

Groups	Plasma	Brain	Liver	Kidney
CG	134.84±4.06***	118.85±7.40***	43.48±2.93***	48.02±2.31***
TG	300.98±8.04	146.58±2.29	94.06±2.84	66.93±2.63
C1	288.26±5.33 ns	136.93±4.33*	85.96±1.75 ns	60.63±4.70 ns
C2	286.73±8.43 ns	134.76±3.53**	83.64±3.29*	57.30±1.09**
C3	282.83±11.00 ns	138.59±6.32 ns	80.96±5.01**	60.38±7.55 ns
C4	260.31±22.38***	123.59±2.15***	57.13±4.58***	51.72±1.94***
C5	284.81±6.24 ns	140.26±5.09 ns	83.79±8.64*	62.05±5.80 ns
C6	263.31±16.69***	141.93±5.61 ns	85.46±10.66 ns	63.72±5.09 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.

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

Table 5:- Effect of synthesized compounds on glutathione transferase (GST) level in cadmium exposed animals after 21 days of treatment.

Groups	Plasma	Brain	Liver	Kidney
CG	16.15±1.45***	10.09±0.38***	20.50±0.77***	34.09±0.72***
TG	19.71±2.36	14.36±0.83	76.79±1.49	44.52±1.25
C1	17.11±1.03*	13.00±0.66 ns	69.15±4.26 ns	41.34±1.25**
C2	17.44±1.05*	12.45±0.60**	69.46±4.49 ns	41.84±1.02*
C3	18.11±0.89 ns	12.85±0.83 ns	72.83±3.24 ns	41.01±1.80**
C4	16.28±0.58***	10.43±0.43***	53.96±8.39***	39.84±0.73***
C5	17.44±0.67*	12.83±1.34*	70.12±7.70 ns	41.67±0.82*
C6	17.61±0.93 ns	12.67±1.31*	65.62±9.69*	43.51±2.39 ns

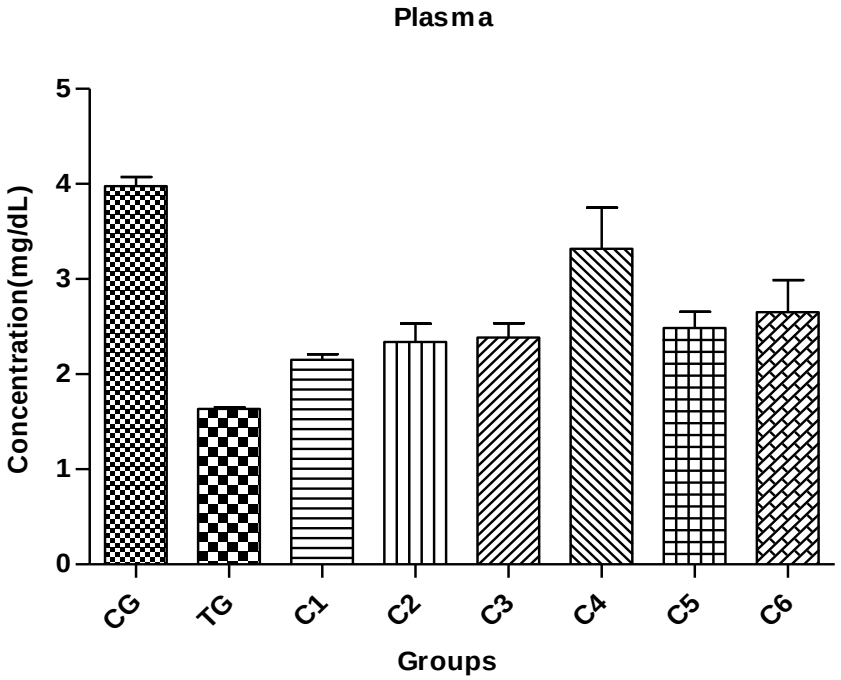
Values are represented Mean ± SD (n=6), significantly different at ***P<0.001, **p<0.01, *p<0.05 and not significant ns>0.05 in comparison to toxic control group.

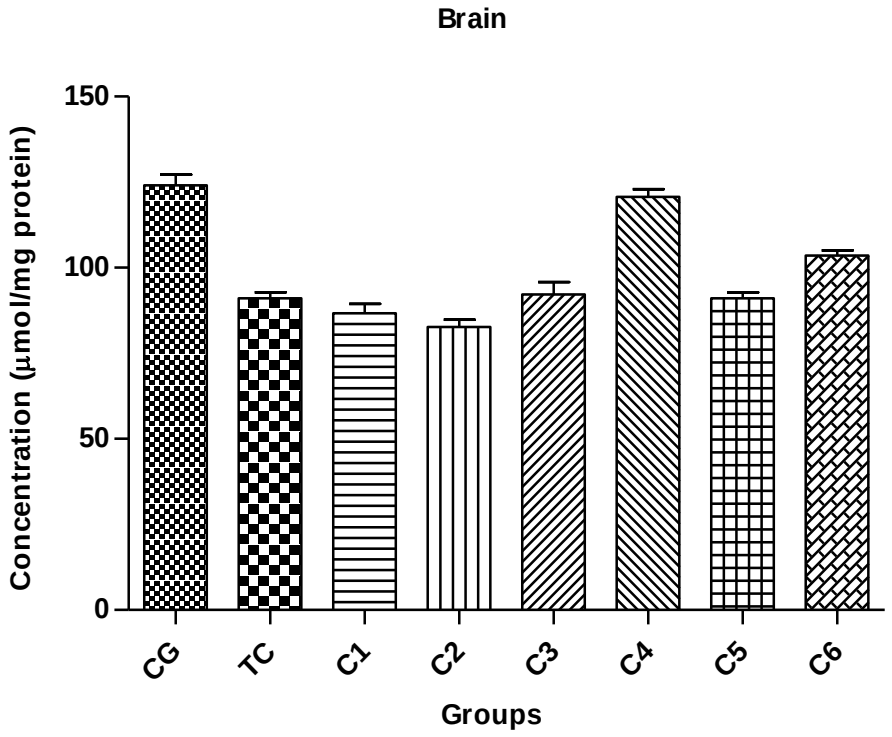
GST level was expressed in the plasma (mg/dL) whereas in the tissues (µmol/mg protein).

Total thiol

Total thiol level was significantly lowered in plasma, brain, 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 (Table – 6.5). This rise in the level of total thiol was more significant with the C4 synthesized compound.

The level of total thiol was 3.31±0.97 mg/dL in plasma, 120.72±4.91 µmol/mg protein in brain, 4.35±0.69 µmol/mg protein in liver and 68.20±1.03 µmol/mg protein in kidney, which was comparable with toxic control group i.e. 1.64, 90.08, 2.44 and 57.15 in plasma, brain, liver and kidney, respectively. However, total thiol level of α-mercapto-β-aryl acrylic acid derivatives was 2.15, 87.71, 3.22 and 63.85 for C1, α-Mercapto-β-thienyl acrylic acid; 2.31, 82.79, 3.37 and 59.85 for C2, α-Mercapto-β-(p-methoxyphenyl) acrylic acid; 2.38, 92.20, 2.77 and 58.68 for C3, α-Mercapto-β-(p-dimethyl amino phenyl) acrylic acid; 2.48, 91.06, 2.97 and 57.18 for C5, α-Mercapto-β-(o-nitrophenyl) acrylic acid; and 2.64, 104.55, 3.27 and 52.21 for C6, α-Mercapto-β-[ethyl-2-(amino)-4-thiazole glyoxylate] acrylic acid in plasma, brain, liver and kidney, respectively as shown in the figure-1.




Brain
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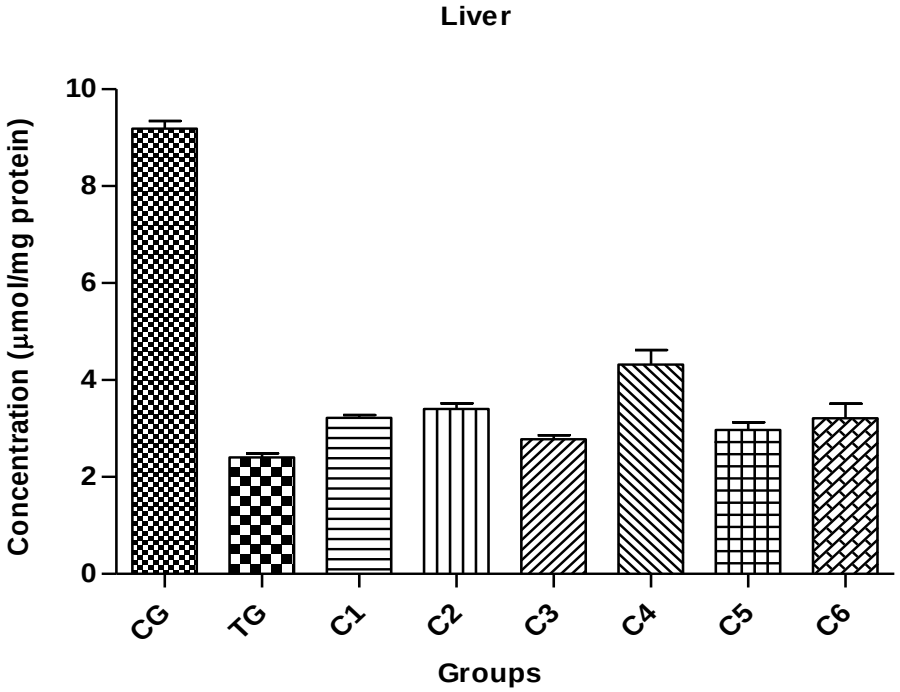
100
50
0
CG
TC
C1
C2
C3
C4
C5
C6
Groups


Liver
Concentration (µmol/mg protein)
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8
6
4
2
0
CG
TG
C1
C2
C3
C4
C5
C6
Groups

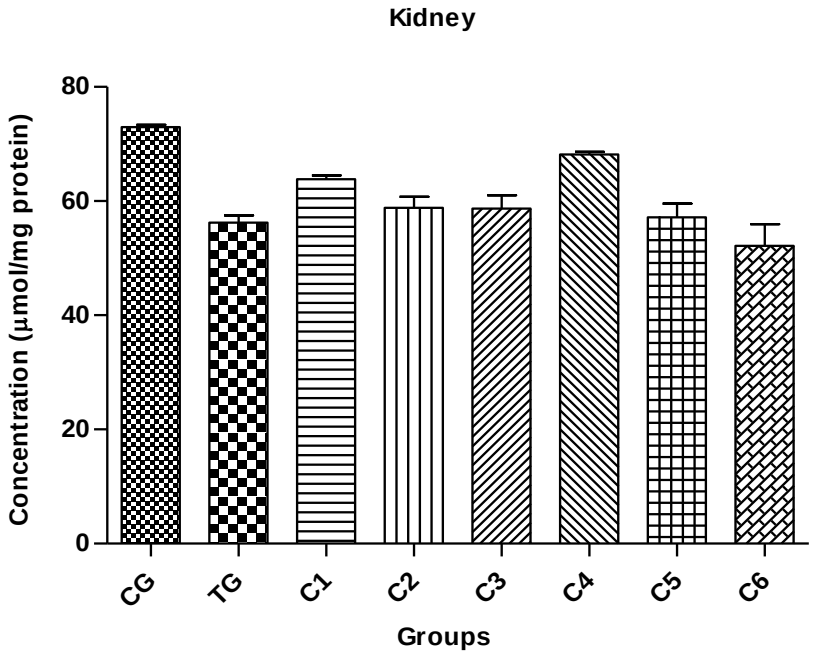

Figure 1:- Total thiol in cadmium exposed animals after 21 days of treatment.

Lipid peroxidation (MDA and MPO)

Reduction in the level of MDA was more significant with C4 synthesized compound among other synthesized compounds.Similarly, the reduction in MPO level by C4 compound. It has reported that degradation of polyunsaturated fatty acids in cell membranes to exposure of Cd, results in the destruction of membranes and the formation of thiobarbituric acid reactive species, MDA, or conjugated dienes as indicators of lipid peroxidation[27]. The increased MDA levels in animal tissues is an indicator of over accumulation of lipid peroxides. It caused depletion of functional thiol (-SH) groups in several antioxidant enzymes. It was restored by treatment with synthesized α-mercapto-β-aryl acrylic acid derivatives. Effect of synthesize compounds on MDA level and MPO level shown in Table – 6 and Table-7.

Table 6:- Effect of synthesized compounds on MDA level in cadmium exposed animals after 21 days of treatment.

Groups	Plasma	Brain	Liver	Kidney
CG	161.10±4.35***	84.2±3.95***	311.21±9.01***	3.79±0.31***
TG	274.83±7.55	117.26±3.48	386.65±13.01	0.91±0.04
C1	263.40±6.88 ns	100.39±9.22*	374.90±8.38 ns	1.86±0.38 ns
C2	258.35±6.99*	101.78±7.51*	365.07±8.39*	2.36±0.71**
C3	267.16±10.62 ns	102.06±8.15*	366.74±8.43 ns	1.53±0.48 ns
C4	192.90±2.61***	90.39±1.33***	333.24±10.32***	2.81±0.82***
C5	255.97±12.44**	110.39±7.96 ns	356.40±16.93**	1.36±0.92 ns
C6	267.45±6.93 ns	122.06±14.89 ns	368.24±14.62 ns	1.21±0.70 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.

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

Table 7:- Effect of synthesized compounds on MPO level in cadmium exposed animals after 21 days of treatment.

Groups	Plasma	Brain	Liver	Kidney
CG	5.85±0.21***	23.57±0.85***	16.36±0.86***	10.86±0.88***
TG	8.26±0.34	32.60±1.10	40.54±1.15	17.98±0.85

C1	7.51±0.69 ns	29.70±3.89 ns	38.20±1.85 ns	15.90±2.46 ns
C2	7.34±0.21*	27.20±1.48*	36.70±1.36**	13.90±0.72**
C3	7.06±0.26**	30.53±3.62 ns	38.37±0.48 ns	15.07±1.60*
C4	6.84±0.35***	25.8±1.70**	23.37±1.34***	12.74±0.52***
C5	7.22±0.42*	30.36±3.01 ns	37.37±1.14**	15.57±2.33 ns
C6	8.06±0.99 ns	30.23±4.82 ns	38.20±2.49 ns	16.90±2.47 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.

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

Discussion:-

In the present study, six different α-Mercapto-β-acrylic acid derivatives were prepared using stearylmethylene 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 as chelating agent for complexation with Cd. The toxicities in blood and tissues 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. Total thiol level was significantly lowered in plasma, brain, liver and tissue of the cadmium exposed group in comparison to the control group. 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 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. Conversely, there were highly significant increases in Malondialdehyde (MDA) and Myeloperoxidase (MPO) levels in plasma and tissues of the $CdCl_2$ exposed group as compared to the control group after 21 days. Treatment with synthesized acrylic acid derivatives; the MDA and MPO parameters were significantly restored in plasma and tissues of the treated group as compared to the toxic group. Reduction in the level of MDA was more significant with C4 synthesized compounds among other synthesized compounds. Similarly, the reduction in MPO level by C4 compound was 6.84±0.35 in plasma, 25.8±1.70 in the brain, 23.37±1.34 in liver and 12.74±0.52 in the kidney, which was comparable with toxic control group i.e. 8.26, 32.60, 40.54 and 17.98 in plasma, brain, liver, and kidney respectively.

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 antioxidant activity was more significant with the C4 group. It increased XO activity in plasma (260.31 mg/dL), brain (123.59 μmol/g tissue), liver (57.13 μmol/g tissue) and in kidney (51.72 μmol/g tissue), which was comparable with toxic control group i.e. 300.98, 146.58, 94.06 and 66.93 in plasma, brain, liver, and kidney, respectively. On the other hand, it decreased GST level in plasma (16.28mg/dL), brain (10.43 μmol/g tissue), liver (20.50 μmol/g tissue) and in kidney (34.09 μmol/g tissue), which was comparable with toxic control group i.e. 19.71, 14.36,76.79 and 44.52 in plasma, brain, liver, and kidney respectively.

Conclusion:-

Treatment with α-mercapto-β-aryl acrylic acid derivatives decreased the cadmium level in blood and tissues in all groups. Total thiol level was significantly lowered in plasma, brain, liver and renal tissue of the cadmium exposed group in comparison to the control group. 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 antioxidant activity was more significant with the C4 group. So, without any major side effect, α-Mercapto-β-acrylic acid derivatives were found effective in reducing Cd toxicity 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 synthesized α-Mercapto-β-acrylic acid derivatives are effective against Cadmium toxicity,

Declaration

The authors declared no conflict of interest.

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