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AMELIORATING EFFECT OF CINNAMON CONJUGATED GOLD NANO PARTICLES ON CADMIUM INDUCED TOXICITY OF LIVER IN RATS

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ABSTRACT Cadmium chloride ($CdCl_2$) is a chemical which is used in many industries like electroplating, dyeing, photocopying etc. It is known for its toxic effects and it is a potent cancer inducing chemical. This study is taken on to formulate a biocompatible nano compound which can treat the damages caused by $CdCl_2$. Nano technology being the most promising technology used in bio-medical fields and Gold nano particles which can effectively target the damaged cells and deliver the drug coated on its surface, is being undertaken in the present study using cinnamon as the herbal component. Preparation and characterization of Biocompatible nano particle were undertaken by UV visible spectroscopy, EDX, SEM and FTIR analysis. Four groups of rats were maintained as control, $CdCl_2$ treated, $CdCl_2$ with gold nano particle and $CdCl_2$ conjugated gold nano particle given as subcutaneous doses. Biochemical parameters like serum transaminases, alkaline phosphatase, total protein and bilirubin were analyzed. to know the intensity of the damage caused by $CdCl_2$ and to infer the effect of cinnamon conjugated gold nano particles. Hypothesis of the study has been proven, giving a scope for further studies.

KEYWORDS : Cadmium chloride, green synthesis, gold nano particle, cinnamon, conjugated gold nano particles

INTRODUCTION:

Cadmium is an inorganic toxicant which is of serious environmental concern. The extremely long biological half life makes it a cumulative toxin. Cadmium primarily gets accumulated in the liver and kidney, as they are the sites of purification (Waalkes, 2003). Cadmium chloride used in the present study, is a white crystalline compound of Cadmium and Chlorine, highly soluble in water and slightly soluble in alcohol. It is being widely used in electroplating, dyeing and photocopying industry. Lung cancers were induced in rats exposed to cadmium chloride aerosols for 18 months (Takenaka, *et al*.,2006). Leydig cell tumours of the rat testis induced by a single subcutaneous injection of cadmium chloride were studied by electron microscope and histochemical methods in 1970s (Reddy *et al*., 1973).

Liver is the largest gland of the body, functioning as a crucial hub of numerous physiological processes. These include macronutrient metabolism, blood volume regulation, immune system support, endocrine control of growth signaling pathways, lipid and cholesterol homeostasis and the breakdown of Xenobiotic compounds, including many current drugs (Bischoff *et al*., 2018). There are many studies on the effects of cadmium chloride on liver cells. In a study with chick embryo, the metal ion, Cd, was found to have severe damaging effect on chick embryonic liver. It has been suggested that exposure to cadmium chloride during pregnancy can cause damages to the embryo (Keeedam *et al*., 2012). In many studies the liver injury is due to acute toxicity dominated by apoptosis and necrosis. The injury of liver can be preliminarily tested with the serum analysis of liver transaminases. Liver transaminases (AST and ALT) are useful biomarkers of liver injury in a patient with some degree of intact liver function (Johnston, 1999), they are raised in acute liver damage, but are also present in red blood cells, and cardiac and skeletal muscle, so is not specific to the liver. Alkaline phosphatase is another enzyme found in many tissues, elevated levels of which are commonly related to liver diseases. Variation in total protein and bilirubin can also be indicators of liver damages.

Nature is a treasure trove of many phytochemicals which possess medicinal properties. One such phytochemical is Cinnamaldehyde obtained from the barks of Cinnamon. Cinnamon is a spice obtained from the bark of trees from the genus Cinnamomum. Cinnamomum Zeylanicum. called "true cinnamon tree" or Ceylon cinnamon tree is a small evergreen tree belonging to the family Lauraceae, native to Sri Lanka. The old botanical synonym for the tree—Cinnamomum zeylanicum—is derived from Sri Lanka's former name, Ceylon. Cinnamon (Dalchini) is a herb traditionally used by many ancient cultures, for a variety of ailments including gastrointestinal problems, urinary infections, relieving symptoms of colds and flu and have remarkable anti-fungal and anti-bacterial properties. Some studies have shown that Cinnamon helps people with diabetes, metabolise sugar better (R.A Anderson *et al.*, 2004). Anti-tumor effect of cinnamon extracts has been investigated and proved in mouse melanoma models (Kwon *et al.*, 2010).

This research work is an attempt to combine the potentials of naturally obtained phytochemical and the nano technology, which is the latest and the most promising scientific technology of this era. Gold nano particles have been used in combination with the natural cinnamon. The synthesis of gold nanoparticles and nano conjugates has been done by many researchers and they have been used in many diagnosis and treatments. But the attempt of using cinnamon conjugated nano particle in treatment of cadmium induced toxicity, is the first of its kind.

MATERIALS AND METHODS

Selection of animal model:

The animal model chosen for the experiment is white Albino Wistar rats, as they are easily available and these are more active, easy to maintain, optimum size to handle and attains maturity within 12 weeks. After getting the ethical clearance, the rats were maintained at PSG animal house for the experiments.

Cadmium chloride:

For this study Cadmium chloride was commercially purchased from Sigma Aldrich. A solution of cadmium chloride was prepared by dissolving 2mg of Cadmium Chloride in 10ml of saline and weekly dosage of 0.1ml per 100g body weight were given.

PREPARATION OF GOLD NANO PARTICLES:

Synthesis is by green synthesis method using 2mM solution and 1mM solution of chloroauric acid and distilled water.

Anhydrous Cloroauric acid (HAuCl4) was purchased from Sigma Aldrich. 5ml of plant extract were added to 10ml of 2mM and 1mM solutions which were kept in darkness for 24 hours. Then the samples were identified and characterized for nanoparticles using UV – Vis spectroscopy, FTIR, XRD and SEM.

CHARACTERISATION OF GOLD NANO PARTICLES: UV- VISIBLE SPECTROSCOPY

The formation of gold nanoparticles was identified by scanning the solution containing gold nanoparticles at the wave length ranging from 400 – 700nm using Shimadzu UV – 1601 spectrophotometer.

ENERGY DISPERSIVE X-RAY (EDX)

Energy-dispersive X-ray (EDX) analysis was carried out using JEOL JEM 2100 high resolution transmission electron microscope to confirm the presence of gold in the particles as well as to detect other elementary compositions of the particles.

SCANNING ELECTRON MICROSCOPE (SEM)

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm – 1. 0.0007g sample was pressed with 0.2000g of KBr for IR spectroscopy Shimadzu, Japan.

CONJUGATION WITH CINNAMON:

Nano conjugation of biosynthesized nano particle with cinnamon extract was done. The prepared gold nanoparticles which is dispersed in the liquid medium was centrifuged at 12000rpm for 20 minutes. Gold nano particles were collected as pellet. To the pellet 500μl of extract was added. Solution was incubated at room temperature for 24hours, after which it was centrifuged at 4500rpm again to separate the conjugated pellet and was resuspended in phosphate buffer. (Sinha R *et al*., 2006)

ANALYSIS OF CONJUGATED NANO PARTICLES: FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm–1. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Shimadzu, Japan.

UV- VISIBLE SPECTROSCOPY

The formation of the conjugated gold nanoparticles were identified by scanning the solution containing cinnamon conjugated gold nanoparticles at the wave length ranging from 400 – 700nm using Shimadzu UV – 1601 spectrophotometer.

EXPERIMENTAL SETUP:

The rats were grouped into four groups of four rats each and maintained using commercial feed and water was given *adlibitum*.

GROUP A:

Control group rats given 0.1 ml saline per 100 gram body weight as a weekly dose orally.

GROUP B:

Treatment group given 0.1 ml per 100gm body weight weekly as subcutaneous injections for four weeks.

GROUP B1:

Supplementation group given subcutaneous injections of gold nano particles with dosage of 0.1ml per kg body weight, weekly for 4 weeks, This was after giving injections of 0.1 ml cadmium chloride, per 100 gm body weight for the first four weeks as subcutaneous injections.

GROUP B2:

Second supplementation group were given subcutaneous injections of conjugated gold nano particles. Dosage was 0.1ml per kg body weight, weekly for 4 weeks. This was after giving injections of cadmium for the first four weeks.

The rats were maintained for eight weeks in total. Blood was collected after the stipulated time and send for biochemical analysis.

ESTIMATION OF SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT)

The concentration of SGOT was estimated in the serum by the method of Reitman and Frankel (1957).

ESTIMATION OF SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT):

The concentration of SGPT was estimated in the serum by the method of Reitman and Frankel (1957).

ESTIMATION OF ALKALINE PHOSPHATASE (ALP)

Alkaline phosphatase is determined by colorimetric method of Bowers and Mc Comb (1988).

ESTIMATION OF BILIRUBIN

The amount of bilirubin is determined by Chancy and Marbagh, (1962).

ESTIMATION OF TOTAL PROTEIN

The total protein concentration of blood serum was estimated by the method of Lowry *et al.*, (1951).

Statistical Analysis

Results obtained were tabulated. Statistical analysis was carried out using Dunnet's "t" test. Any significant variation between the control and treated groups were recorded (Steele and Torrie, 1960).

RESULTS:

GAS CHROMATOGRAPHY RESULTS ON ANALYSIS OF POWDERED CINNAMON BARK.

Cinnamon bark powder was analyzed using gas chromatography to find out the components (table 1).Equipment used was THERMO GC - TRACE ULTRA VER: 5.0,THERMO MS DSQ II and the column was MS CAPILLARY STANDARD NON - POLAR COLUMN. Carrier gas was He with a flow rate of 1.0ml/min.

Table 1: Components present in the cinnamon bark powder analyzed through gas chromatography

No.	Name of the compound	Mol. Formula	MW	Compound Nature
1	ç-Terpinene	C10H16	136	Alkaloids
2	Cinnamaldehyde	C9H8O	132	Phenyl group
3	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (CAS)	C12H14O3	206	Alkaloids
4	Cinnamaldehyde dimethylacetal	C11H14O2	178	Aldehydes
5	Cis-2-Methoxycinnamic acid	C10H10O3	178	Carbonyl compounds
6	Caryophyllene	C15H24	204	Essential oils
7	Ortho methoxy cinnamic aldehyde	C10H10O2	162	Bio active compound

8	Tetradecanal	C14H28O	212	Myristic acid
9	9-Octadecena	C18H34O	266	Aldehyde
10	Trans-Z-α-Bisabolene epoxide	C15H24O	220	Sesquiterpene oxide
11	Campesterol	C28H48O	400	Steroid

CHARACTERIZATION OF NANO PARTICLES: COLOUR CHANGE

The first proof for synthesis of nano particles was the appearance of purple color. The change in colour was obtained within few minutes of addition of plant extract. After 24 hours, the color was intense with a powdered appearance of particles in solution (fig 1).

Fig 1: Appearance of purple color in solutions of 1mM and 2mMHAuCl$_4$indicating the synthesis of gold nanoparticles

UV – VISIBLE SPECTROSCOPY:

The formation of gold nanoparticles was identified by scanning the solution containing gold nanoparticles at the wave length ranging from 400 – 700nm using Shimadzu UV – 1601 spectrophotometer.

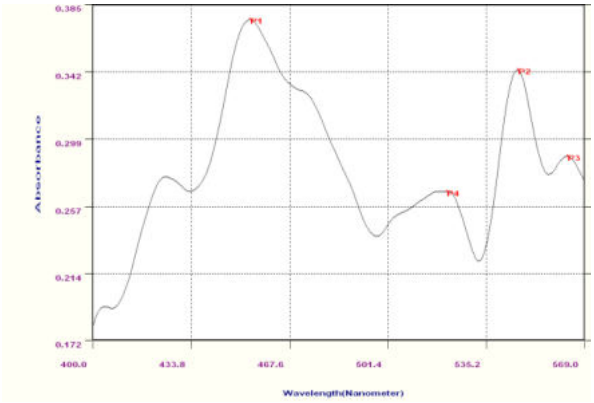

Fig 2: UV - Vis spectrum of 1mm gold nanoparticles

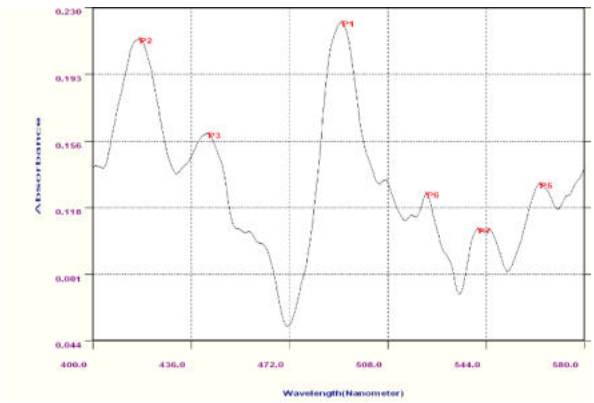

Fig 3: UV – Vis spectrum of 2mm gold nanoparticles.

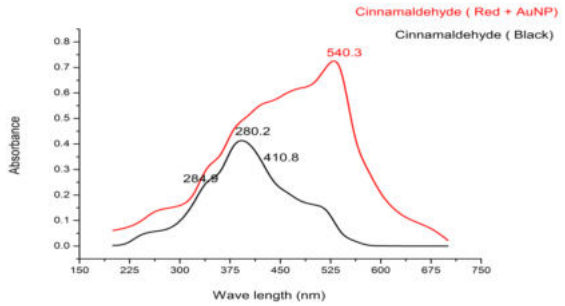

Fig 4: UV Vis spectrum of conjugated gold nano particles

EDX AND SCANNING ELECTRON MICROSCOPY ANALYSIS:

Energy-dispersive X-ray (EDX) analysis was carried out using JEOL JEM 2100 high resolution transmission electron microscope to confirm the presence of gold in the particles as well as to detect other elementary compositions of the particles.Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, the extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.The synthesis of gold nanoparticles using cinnamon extract was confirmed by the characteristic peak obtained in the EDX image and the structural view under scanning electron microscope.

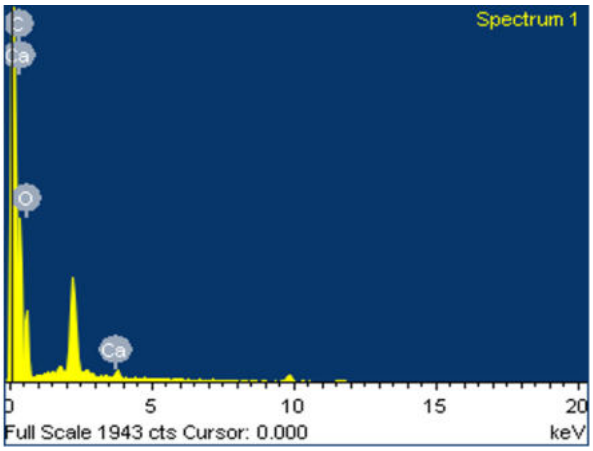

Fig5: EDX image with four dominant peaks for C, Ca, O and gold

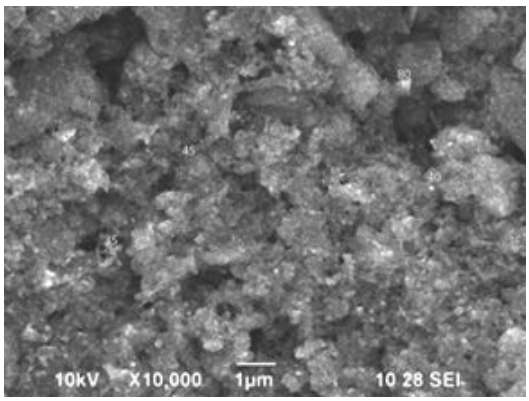

Fig 6: SEM image showing NPs in the range of 35 – 80 nm

FTIR ANALYSIS

The FT-IR investigations were carried out with a Scimitar Series FTS 2000 Digilab spectrophotometer in the range of middle infrared of 4000-400 cm–1. 0.0007 g sample was pressed with 0.2000g of KBr for IR spectroscopy Shimadzu,

Japan. The number of scans 16 and the resolution of 4 cm-1 characterized these measurements. The peaks show the presence of gold nanoparticles. Absorbance bands are observed in the region of 1200 -1800 cm^{-1}. The FTIR spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins of plant extract has strong ability to bind metal, and most possibly might have formed a layer on the gold nanoparticles. Similar peaks were reported by other researchers also.

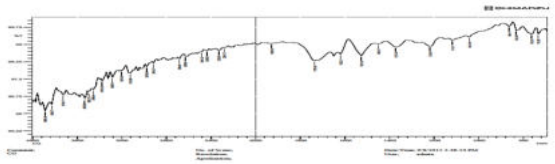

Fig 7: FTIR analysis of the solution containing gold nanoparticles

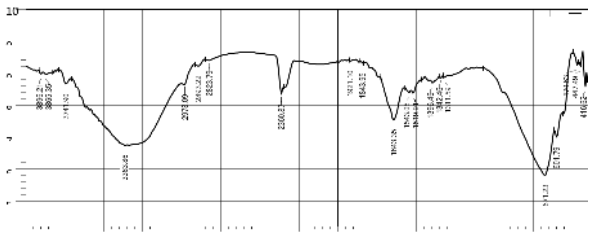

Fig 8: FTIR Analysis of the solution containing conjugated gold nano particles

	Item	Value
2	Sample name	Cinnam +AuNP
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	45

BIOCHEMICAL ANALYSIS

Administration of cadmium chloride to rats was observed to significantly increase the activities of SGOT, SGPT and ALP in serum. On contrast, supplementation with gold nano particles and conjugated gold nano particles were seen to significantly reduce the activities of SGOT, SGPT and ALP. Between the two supplementations, supplementation with gold nano particles seems to be more beneficial. The level of total bilirubin was observed to be significantly increased

TABLE:2: Effect of cdcl2 , gold nanoparticles, conjugated gold nano particles on serum transaminases, ALP, Total bilirubin and protein. The table shows the variation of the amount of the parameters like liver transaminases, alkaline phosphatase, total bilirubin and total protein.

GROUPS	PARAMETERS				
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TOTAL BILIRUBIN (mg/dl)	TOTAL PROTEIN (gm/dl)
GROUP A	119.2 ± 1.241	40.2 ± 1.655	222 ± 1.517	0.256 ± 0.012	6.34 ± 0.107
GROUP B	324.4 ± 1.208*	262.6 ± 1.536*	988 ± 2.429*	0.9 ± 0.007*	6.04 ± 0.102
GROUP B1	222.2 ± 0.860*α	103.6± 0.748*α	210 ± 1.140*α	0.93± 0.014*	7.86± 0.117*α
GROUP B2	291.4 ± 3.076*α	066 ± 1.703*α	252.6 ± 1.536*α	1.20± 0.016*α	5.76± 0.081*α

Values are expressed as mean ± S.E.M of four rats.
*Significance at 5% level of Group A Vs All groups, αSignificance at 5% level of Group B Vs Group B1, B2.

Group A - Normal Control, Group B- Cadmium induced 4 weeks, Group B1- Cadmium induced treated with gold nanoparticles and Group B2 – cadmium induced and treated with conjugated nano particles

DISCUSSION

Cadmium is the most dangerous occupational and environmental toxin, found in drinking water, atmospheric air and even in food. The toxicity of this metal lies indirect action of free Ca ions not bound with metallothionein on one hand, and forming reactive radicals which change the structure and function of many organ systems on the other hand (Jarup *et al*, 2000).

In the present study, administration of Cadmium chloride was observed to raise the activities of GOT, GPT and ALP in the serum of rats, with an increase in total bilirubin and slight decrease in total protein. Ghonin *et al*., 2017, have also reported about the hepatotoxic effect of Cadmium expressed as elevated ALT,AST and ALP levels. These increased levels ca be attributed to hepatocellular degeneration as a result of Cadmium induced oxidative damage into liver. Both aminotransferases are mainly concentrated in the liver. ALT is localised solely in the cytoplasm, while AST is present both in cytosol and mitochondria of hepatocytes (Haidry and Malik 2014). The increase in permeability of cell membrane of hepatocytes, due to cadmium induced lipid peroxidation lead to the release of transaminases into the blood. The increase in ALP activity is indicative of hepatic toxicity and biliary obstruction (Naik, 2010).

The increase in total bilirubin level may be due to increased erythrocyte breakdown and separation of haem fromglobin, conversion into bilirubin in spleen and final reduction to bilirubin (Doumas *et al*, 1973).The decrease in serum total protein can be attributed to the impairment of hepatocyte functions causing decrease in cytochrome P450 activity, protein metabolism inhibition in liver (Asagba 2010), as a result of Cd insult. Significant decrease in total protein and significant increase in total bilirubin on cadmium exposure has been reported by Oyinloye *et al*.,(2016). Inducement of severe hepatotoxicity is further expressed by elevated serum levels of AST and ALT, due to loss cellular integrity and leakage of hepatic membrane. Ibiam *et al.*, (2013) have also reported about the significant elevation of ALT, AST, ALP and bilirubin in rats exposed to Cd with a significant decrease in total protein in a dose dependent manner as a result of impaired hepatocyte function.

Nanomaterials and GNPs, in particular are promising in biomedical applications due to their high stability, low toxicity and excellent biocompatibility. When the nanoparticles are given with additional ability to functionalize their surface with various drug molecules, their biomedical applications become manifold. Conjugated nanoparticles are proved to have the ability to enter damaged / tumour induced hepatic cells at a higher concentration in comparison with normal cells. (Tomleasa *et al*).

Nanoparticle based drug delivery systems are proven to have the ability to have a 'controlled – release reservoir', which can safely deliver the therapeutic agents to injury cites or specific cells (AncutaJurj *et al*). The alluring effect of gold nanoparticles conjugated with cinnamon is visible when the results of biochemical assays are observed. The significant decrease in SGOT, SGPT and alkaline phosphatase levels, gives the inference of the ability of the effective targeted drug delivery and biocompatibility of gold nanoparticles. Also the alluring effect of cinnamon, a plant rich with phytochemicals having anticancer properties.

CONCLUSION:

The elevated levels of ALT, AST and ALP indicates that there is

a damage caused to liver tissues. Cadmium chloride is a potent chemical which can cause damages even on exposure to small doses. The alluring effect of cinnamon conjugated gold nano particles is also evident. Long term treatments can significantly bring remedial changes in the damaged tissues.

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