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**Phytochemical analysis of Greentea (*Camellia sinensis*)
and assessment of its scavenging ability on CdCl₂**

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Abstract

Cadmium is a widespread environmental and industrial pollutant, induces severe alterations in the tissues of both animals and humans. Parental administration of cadmium chloride (CdCl₂) result in the lipid per oxidation occurs in kidney, liver and other tissue of rats and mice. Cadmium poisoning is the result of too much exposure to the poisonous mercury resulting in destruction to the immune system and causes many unrelated diseases and affects every system in the body. Aim of the present study was phytochemical analysis of green tea and assessment of its scavenging ability on CdCl₂. To assess that Rats orally administered with cadmium chloride (3.5 mg/kg body weight p.o.) dissolved in 0.01N nitric acid dose for 3 days followed 3 different doses 250 mg/kg, 500mg/kg and 1g/kg Of *Camellia sinensis* dissolved in water simultaneously for 21 days and the effect was studied using the following markers bodyweight, urea, creatinine, cholesterol, TAG, SOD, catalase, vitamin C and vitamin E. The obtained results proved that CdCl₂ elevates urea, creatinine, cholesterol and TAG and decreases SOD, catalase, vitamin C and vitamin E levels and the deviation was nullified by 1g/kg of *Camellia sinensis* and it also confirmed that the optimum dose was found to be 1g/kg. The mechanism of detoxifying action of *Camellia sinensis* is most probably based on their high affinity and ability to form insoluble complexes with cadmium ions. This could be due to efficient chelating activity of *Camellia sinensis* containing flavonoids, polyphenols and other phytoconstituents present in it. It was also supported by earlier findings and proved that *Camellia sinensis* has potent anti-inflammatory and antioxidant capabilities.

Keywords: Cadmium, *Camellia sinensis*, anti-inflammatory and antioxidant capabilities.

Introduction

Most common and harmful heavy metals are aluminium (Al), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg) and nickel (Ni). Both occupationally and environmentally these heavy metals constitute a significant potential threat to human health (Hu, 2000). However, some of them occur in food, water and air

even in the absence of occupational exposure (Atef Al-Attar, 2011) through consumption of plants (Sharma and Agarwal, 2005) constitute a significant potential threat to human health (Hu, 2000), plants and animals have become recognized source of illness worldwide (Lyn Patrick, 2003).

Most of the heavy metals are free radicals that contain one or more unpaired electrons (Halliwell and Gutteridge, 1999) acts in many ways through the production of super oxide anion, the primary ROS and can interact further with other molecules to generate secondary ROS, either directly or prevalently through enzymes or metal catalyzed process (Fridovich, 1986 and Valko *et al.*, 2005). Cadmium intoxication leads to kidney, bone, and pulmonary damages.

Materials and Methods

Chemicals Used

The fine chemicals Alanine, Aspartic acid, Cholesterol, 2,4-dinitro phenyl hydrazine, DAM- TSC reagent, Phosphotungstic acid, Sodium pyruvate, Urea, Sulphuric acid, Albumin, Bilirubin and Adenosine triphosphate (ATP) used for the present study purchased from Merck companies, Mumbai, India. Cadmium chloride Santa Cruz, USA and the rest of the chemicals and biochemical utilized were obtained from local firms (India) and were of analytical grade. Aim of the present study was

Methods

Standard methods were used for the estimation of factors, activity of various enzymes.

Laboratory animals

Wister strain *albino* rats weighing 180-220g were used. The animals were housed in spacious cages under hygienic condition and maintained on commercial diet containing 21% protein, 5% lipid, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 55% nitrogen free extract and enriched with vitamins as well as minerals. It was supplied by the "Hindustan Lever limited", Mumbai, under the trade name "Gold mohur Feeds". Water was provided *ad libitum*. The rats were acclimatized in animal house for ten days before starting the experiment.

Collection of the sample

The fresh leaves of *Camellia sinensis* was collected from the dense tea estate garden at Ooty, Coimbatore district, Tamil Nadu, INDIA.

Preparation of the extract

The leaves of fresh samples were cleaned and washed under running tap water (Tariq and Reyaz, 2012). The samples were dried in the oven at 37°C for 6 days. After drying the samples were weighed and blended with warring blender and soaked with methanol [in ratio methanol: plant (6:1)] for 2 days and filtered using Whatman No. 1 paper. The methanol was completely removed by vacuum evaporator at

50°C till it gave a viscous mass. The crude extracts were weighed and stored at 4°C before analysis.

Preparation of CdCl₂ and induction of multiple organ failure:

Required quantity of CdCl₂ was taken and dissolved in 0.1N HNO₃. Rats administered orally by stomach tube with cadmium in the form of cadmium chloride at the concentration of 3.5mg/kg b.wt for 3 days (Venkatesan *et al.*, 2011).

Experimental Design

A total number of 36 rats were taken for this present study, considered the carefully their age, sex (male) and weight.

Group A: (Control): Rats orally administered with 0.01N nitric acid dose (0.5ml/day) for 21 days.

Group B: Rats administered with *Camellia sinensis* dissolved in water (1g/kg p.o.) for 21 days.

Group C: Rats orally administered with cadmium chloride (3.5 mg/kg body weight p.o.) dissolved in 0.01N nitric acid dose for 3 days. The dosage of cadmium chloride was determined according to Venkatesan, *et al.*, (2011).

Group D: Rats orally administered with cadmium chloride (3.5 mg/kg body weight p.o.) dissolved in 0.01N nitric acid dose for 3 days followed *Camellia sinensis* dissolved in water (250 mg/kg p.o.) simultaneously for 21 days.

Group E: Rats orally administered with cadmium chloride (3.5 mg/kg body weight p.o.) dissolved in 0.01N nitric acid dose for 3 days followed *Camellia sinensis* dissolved in water (500 mg/kg p.o.) simultaneously for 21 days.

Group F: Rats orally administered with cadmium chloride (3.5 mg/kg body weight p.o.) dissolved in 0.01N nitric acid dose for 3 days followed *Camellia sinensis* dissolved in water (1g/kg p.o.) simultaneously for 21 days.

Preparation of Serum and Plasma

At the end of the experimental period (21 days), all the animals were anesthetized within intramuscular injection of ketamine (75 mg/kg b.wt.) and sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. Blood was collected with anticoagulant and centrifuged (2000 xg for 20 min) to separate plasma. The tissues were dissected out, weighed, minced and homogenized (10% w/v) in Tris-HCl buffer (0.1M; pH 7.4) and centrifuged at 3000 xg for 20 min at 4°C. The resultant supernatant was used for the analysis.

Statistical Analysis

The values were expressed as mean value (n=6) of + S.E.M, The *in vivo* experimental data were analyzed using one way analysis of variance by the Duncan's Multiple comparison test to determine the level of significance (p) and $p < 0.05$ was considered as statistically significant.

Identification of Leaves

The leaves belongs to the Kingdom-Plantae, Order-Ericales, Family-Theaceae, Genus-Camellia, Species sinensis.

Qualitative analysis of phytochemicals

The extract showed the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins and catecholic tannin by changing the colour of the solution to yellow, white, green bluish, blue, green black respectively. While indicated the absence of terpenoid, saponins, and glycosides as there was no colour change in the solution with respect to them. They were analysed by using Standard procedures described by Harborne,(2000).

Total Contents

The total phenolic content in one gram of leaf extracts was found 0.7grams while the total flavonoidic content was 14mg/gram of leaf extract. The reducing power of *Camellia sinensis* were 0.11grams/gram of leaf extract.

Qualitative Analysis

The Fourier Transform Infrared Spectroscopy (FTIR) Qualitative analysis of *Camellia sinensis* obtained was analyzed and interpreted with a chart for characteristics infrared absorption frequencies of organic functional groups and carbonyl containing

functional groups which showed the presence of alkene, alcohol, ester, amine acid, alkane, aromatic alkane, nitro compounds, aromatic amide, alkene amide, carbonyl anhydride, alkane hydroxyl group ketone, aromatic amine, alcohol amine and alcohol (table 1).

The chemical compositions and antioxidant activities of green tea with different plucking periods were determined and the resultant data were subjected to correlation analysis to find the important factors contributing to the antioxidant activity. Additionally, we have re-examined the apparent relationship between individual catechins and antioxidant activity using individual catechin standards with Trolox to verify it. The results show that the antioxidant activities of green teas are dependent on their chemical composition and especially on the presence of *cis*-catechins, found in high contents in relatively old leaves. As such, these findings suggest that the leaf age-dependent changes in catechin composition have the potential to increase the antioxidant activity of green tea and they may possibly be controlled by the agronomic conditions under which the tea is grown. It is expected that the results obtained in the present study will provide a broader understanding of the bioactive compounds of green tea, and enable more informed decisions to be made regarding the plucking policies of tea leaves.

Results and Discussion

Table 1: The extract showed the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins and catecholic tannin by changing the colour of the solution to yellow, white, green bluish, blue, green black respectively. While indicated the absence of terpenoid, saponins, and glycosides as there was no colour change in the solution with respect to them.

Table.1 Phytochemicals study

| S.no | Test | Color observed | Inference |
|------|--------------------|--------------------|--------------------------------|
| 1 | Alkaloid | Yellow ppt | Presence of Alkaloid |
| 2 | Terpenoid | No colour change | Absence of terpenoid |
| 3 | Steroid | Green bluish color | Presence of Steroid |
| 4 | Gallic tannin | Blue color | Presence of Gallic tannin |
| 5 | Cathecholic tannin | Green black color | Presence of Cathecholic tannin |
| 6 | Saponins test | No colour change | Absence of saponin |
| 7 | Total phenols | Blue color | Presence of Total Phenols |
| 8 | Total flavonoids | White color | Presence of Total flavonoids |
| 9 | Glycoside | No color change | Absence of Glycoside |

The plant materials have shown the antimicrobial activities against various pathogenic microorganisms therefore consumption of tea has been associated with reduced risk of major diseases (Robinson.,1997). The beneficial effects of the tea have been attributed to the strong antioxidant activity due to the phenoloic compounds (Jang., 2007)]. The carotenoids, flavonoids, benzoic acid, ascorbic acid, tocotrienols, cinnamic acid, folic acid, tocopherols are some antioxidants produced by the plants for their substance (Bajpai ., 2005).

They also serve in plant defense mechanisms to counteract reactive oxygen species in order to survive

and prevent molecular damage and caused by microorganisms, insects, and herbivores (Akowuah .,2005)

Table 2:-Depicts the changes in the body weight of control and experimental rats, the weights were decreased in the cadmium chloride alone administered rats significantly ($p < 0.05$) than the control rats. On the other hand rats administered with *Camellia sinensis* and $CdCl_2$ showed no considerable weight loss from their initial weight and their weight retained significantly ($p < 0.05$) than $CdCl_2$ alone treated rats.

Table. 2 Effect of $CdCl_2$ and *Camellia sinensis* on control and experimental rats

| Parameters | Control | <i>Camellia sinensis</i> (1g/kg) + | $CdCl_2$ | $CdCl_2$ + <i>Camellia sinensis</i> (250mg/kg) | $CdCl_2$ + <i>Camellia sinensis</i> (500mg/kg) | $CdCl_2$ + <i>Camellia sinensis</i> (1g/kg) |
|----------------------|-------------|------------------------------------|-------------|--|--|---|
| 0 th day | 181 ± 10.43 | 188 ± 11.45 | 179 ± 12.84 | 181 ± 09.97 | 180 ± 10.46 | 181 ± 10.26 |
| 7 th day | 189 ± 11.32 | 201 ± 11.86 | 155 ± 11.03 | 182 ± 11.64 | 184 ± 11.63 | 184 ± 12.36 |
| 14 th day | 210 ± 12.01 | 212 ± 9.98 | 147 ± 9.07 | 184 ± 10.98 | 186 ± 12.03 | 187 ± 11.46 |
| 21 st day | 218 ± 12.36 | 228 ± 12.09 | 138 ± 9.21 | 186 ± 09.88 | 191 ± 9.92 | 193 ± 11.66 |

$CdCl_2$ - Cadmium chloride

Values are expressed in g

Values are means ± S.D for six rats.

Values not sharing a common superscript and differ significantly at $p < 0.05$ (DMRT)

The body weights loss obtained in the $HgCl_2$ administered rats may be due to anyone of the following reasons. The unintentional weight loss may be a result of fat loss, muscle atrophy, fluid loss or a combination of these (National Cancer Institute, 2011; Huffman, 2002), continuing weight loss may be deteriorate into wasting involves a systemic inflammatory response (Yaxley *et al.*, 2012).

The obtained results and evidences corroborated with each other concluded that the body weight loss could be due to decreased protein synthesis and muscle wasting in the rats. Whereas, *camellia sinensis* administered with $CdCl_2$ protected the rats from Cd toxicity and their weight remains similar to that of control rats.

SGOT, SGPT & LDH

Table 3 and depicts administration of $CdCl_2$ showed elevated level of AST, ALT, serum LDH ($p < 0.05$) with respect to the control rats, at the same time simultaneous administration of *Camellia sinensis* with $CdCl_2$ did not notice such elevation from their initial value compared with control and the values remain closure to the control rats significantly ($p < 0.05$). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli, 2006). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in ayurveda recommended for the treatment of the treatment of liver disorder (Chatterjee, 2000).

Table.3 Effect of CdCl₂ and *Camellia sinensis* on control and experimental rats SGOT, SGPT, LDH Level

| Parameters | Control | <i>Camellia sinensis</i> (1g/kg) + | CdCl ₂ | CdCl ₂ + <i>Camellia sinensis</i> (250mg/kg) | CdCl ₂ + <i>Camellia sinensis</i> (500mg/kg) | CdCl ₂ + <i>Camellia sinensis</i> (1g/kg) |
|-------------|------------|------------------------------------|-------------------|---|---|--|
| SGOT | 34.21±1.22 | 36.18±1.98 | 154.29±5.21 | 126.38±3.7 | 49.32±1.98 | 48.65±30.19±1.32 |
| SGPT | 28.89±1.32 | 29.33±1.09 | 120.66±2.21 | 111.12±1.5 | 38.79±1.43 | 38.83±1.43 |
| LDH | 88.24±2.11 | 90.32±3.11 | 169.58±3.11 | 142.13±3.11 | 99.18±2.89 | 7.43±1.39 |

CdCl₂ - Cadmium chloride

Values are expressed in IUL

Values are means ± S.D for six rats.

Values not sharing a common superscript and differ significantly at p< 0.05 (DMRT)

In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Developing countries like India and others have struggled to manage the impact of hepatitis (Sakthipriya, 2011). The enzymes used in the assessment of liver function are AST(SGOT), ALT(SGPT). Increase in SGOT and SGPT reflects cell damage and registers serum enzymes increase 20 times than the normal range in hepatitis

Urea, Creatinine

The kidney is the principal organ targeted by chronic exposure to cadmium. Cadmium nephrotoxicity may follow chronic inhalation or ingestion. Data from human studies suggest a latency period of

approximately 10 years before clinical onset of renal damage, depending on intensity of exposure. However, subtle alterations of renal function have been described after acute exposure in animals, and there are rare reports of renal cortical necrosis after acute high-dose exposure in humans. Most kidney diseases attack the nephrons, causing them to lose their filtering capacity. Damage to the nephrons can happen quickly, often as the result of injury or poisoning.

Table 4 explains that rats administered with CdCl₂ alone showed a significant (p<0.05) increase in the urea and creatinine level compared with the control rats. On the other hand *Camellia sinensis*(1g/kg) and CdCl₂ simultaneous administration protected the rats from the elevation of urea and creatinine and the values remain almost similar to the control rats significantly (p<0.05).

Table 4 Effect of CdCl₂ and *Camellia sinensis* on control and experimental rats urea, creatinine and cholesterol level

| Parameters | Control | <i>Camellia sinensis</i> (1g/kg) + | CdCl ₂ | CdCl ₂ + <i>Camellia sinensis</i> (250 mg/kg) | CdCl ₂ + <i>Camellia sinensis</i> (500mg/kg) | CdCl ₂ + <i>Camellia sinensis</i> (1g/kg) |
|------------------------------|--------------|------------------------------------|-------------------|--|---|--|
| Urea(mg/dl) | 24.12±0.92 | 23.14±1.08 | 54.29±2.81 | 46.28±2.3 | 30.32±1.75 | 29.42±1.53 |
| Creatinine (mg/dl) | 1.18±0.03 | 1.09±0.05 | 1.92±0.08 | 1.42±0.21 | 1.19±0.33 | 1.08±0.33 |
| Cholesterol (mg/dl) | 168.24±2.11 | 158.42±3.28 | 89.28±4.56 | 240.21±3.67 | 173.18±3.32 | 171.28±2.99 |
| Triglycerides (mg/dl) | 3.43 ± 0.29 | 3.78 ± 0.23 | 2.91 ± 0.12 | 3.35 ± 0.26 | 3.18 ± 0.13 | 3.12 ± 0.15 |
| Hb(g/dl) | 12.26 ± 0.36 | 12.06 ± 0.14 | 8.33 ± 0.66 | 10.65± 0.53 | 10.60 ± 0.35 | 11.01 ± 0.19 |

CdCl₂ - Cadmium chloride

Values are means ± S.D for six rats.

Values not sharing a common superscript and differ significantly at p< 0.05 (DMRT)

The elevations of urea and creatinine levels were reported to be proportionate with the severity of renal insufficiency (Kumar, 1994; Long *et al.*, 1998; Cid *et al.*, 2009). Classically, chronic cadmium exposure is associated with progressive renal tubular dysfunction. In the final stages of cadmium nephropathy, glycosuria, wasting of calcium and phosphate, and altered calcium metabolism with secondary effects on the skeleton of osteoporosis and osteomalacia are seen (Roels *et al.* 1999; Jarup *et al.* 2000).

Some experts believe the microproteinuria related to cadmium exposure is not invariably progressive and the level at which cadmium-induced nephropathy becomes progressive and irreversible, even after termination of exposure occurs at urinary. Experts believe that the renal tubular dysfunction associated with cadmium is irreversible (Iwata *et al.* 1993). Cadmium nephropathy is an important determinant of mortality in cadmium workers.

These studies have found that even very low-levels of cadmium may have adverse effects on the kidney. WHO currently states that 200 µg/g levels weight in kidney causes adverse changes in 10% of the population (Stohs *et al.* 2002). In the past, several studies of occupationally and environmentally exposed populations have shown that the threshold for renal damage occurred at urinary cadmium levels of 2-4 nmol/mmol creatinine (Buchet *et al.* 1990); However, the OSCAR study found that those with a urine cadmium level of 1 nmol/mmol creatinine had a threefold risk of increased -1 microglobulin (Jarup *et al.* 2000). It is not known if these early subclinical changes in kidney biomarkers associated with low levels of environmental cadmium exposure have any correlation with continued decline in renal function to clinical levels of concern (Noonan *et al.* 2002).

Liver and kidney are critical organs used to describe and document the effect of pollutants, during exposure, Cd induced toxicity in the liver and kidneys is dependent on hepatic and renal Cd concentrations (TalibHussen Ali., 2008).

Cadmium is transported in the blood and widely distributed in the body but accumulates primarily in the liver and kidneys. Hepatic and renal and toxicity may occurred if toxic Cd level is attained in these organs regardless to exposure periods. Mice treated with Cadmium developed progressive liver alteration characterized by cytoplasmic vacuolation, necrosis, regenerative and eventually followed by cell death. The liver damage has been reported in other mammalian and non mammalian species, in addition necrosis strongly associated with oxidative stress (Bondy.,2003).

Many studies in experimental animals have demonstrated an association between morphological

and functional changes in the kidneys. Exposure to cadmium leads to pathological changes in the kidneys. The morphological changes are initially limited to tubular epithelial cell degeneration, but this is followed by cellular atrophy WHO (WHO.,1992). Roya and Bhattacharya (De Sliva., 1992) reported the accumulation metals in various organs and tissues and excreted through glomerular filtration, but they did not explain the consequences of pathway impact. The present investigation highlights the effect of 5.98 mg/kg b.w. dose of cadmium chloride (CdCl₂) on kidney histopathology showed that was a shrinkage in Bowman,s capsule space followed by enlargement of glomerulus, which may be due an increase in the filtration rate as a mechanism to overcome the toxic effect (Roya., 2006).

Cholesterol, TAG and LDH

Enzyme markers are better indicators of the status of the organ in which it is present. Myocardium contains an abundant concentration of LDH, SGOT are important diagnostic marker enzymes of myocardial infarction, once metabolically damaged, releases its content into the extra cellular fluid (ECF).

Table 4: expresses the administration of Hg in the form of HgCl₂ raised serum cardiac enzyme markers CPK, LDH and as well as biochemical markers cholesterol, TG, FFA, LDL-C with respect to the control group significantly (p<0.05) on the other hand HDL-C and phospholipids level showed a decrease significantly (p<0.05). But the simultaneous administration of *camellia sinensis* (1g/kg) and CdCl₂ brought the deviated values towards the normal level significantly (p<0.05).

Metals accumulated in placental membranes in proportion to their level in the medium. Membrane accumulation of Cd was higher than that of Hg. The cholesterol, phospholipid, and cholesterol-to-phospholipid mole ratios in membranes derived from metal treated explants were unchanged, compared to their respective controls. However, no changes in membrane fluidity were observed in the samples incubated for 6 hr. In conclusion, exposure of placental cells to Hg and Cd caused accumulation of the metals in the membranes and lowered the membrane fluidity, which may affect membrane function and cause damage to the developing fetus (Boadi *et al.*, 1992).

Flavonoids and tri terpene are recently shown to reduce hypertension in experimental animal models, several studies have suggested that high intake of flavonoids may decrease the risk of coronary heart diseases (Mink *et al.*, 2007). Poly phenols may play an important role in protecting the body against cancer and cardiovascular diseases (Mojziso \acute{v} a \acute{e} t *al.*, 1999).

Haemoglobin

Anemia is a medical condition in which the red blood cell count or hemoglobin is less than normal. Anemia is caused by either a decrease in production of red blood cells or hemoglobin, or an increase loss or destruction of red blood cell.

Iron deficiency anemia is a common anemia (low red blood cell or hemoglobin levels) caused by insufficient dietary intake and absorption of iron, and/or iron loss from bleeding which can originate from a range of sources such as the intestinal, uterine or urinary tract (review of numbers of infections).

Table 4: Results denotes, the cadmium chloride administered rats showed significant ($p < 0.05$) reduction in the hemoglobin level compared with the control rats, on the other hand, the trend was different when $CdCl_2$ administered with *camellia sinensis* (1g/kg) in which the hemoglobin level did not deviate much from the normal value and showed significant ($p < 0.05$) protective action compared with $CdCl_2$ alone administered rats.

Normochromic anemia and low blood pressure sometimes also occur (Alfven et al. 2002; Nogawa et al. 2004), and average urinary cadmium level in these patients is 20-30 $\mu\text{g/g}$ -creatinine of cadmium in urine, there is conflicting data that chronic cadmium exposure may cause mild anemia (Ezaki et al. 2003).

Enzymatic antioxidants-SOD, catalase

Table 5: Describes the present study report that cadmium chloride decreases the SOD and CAT levels. However, administration of *Camellia sinensis* (1g/kg) with cadmium no change were observed in SOD and CAT levels compared with control values.

An antioxidant is defined as any substance present at low concentration compared to those of an oxidizable substrate significantly delays or prevents oxidation of those substrates. Antioxidants can inhibit the LPO through competitive binding of oxygen, retardation of propagation step by destroying or binding with free radicals, inhibition of catalysts or stabilization of hydroperoxides (Diplock, 1991; Halliwell and Gutteridge, 1999).

Table .5 Effect of $CdCl_2$ and *camellia sinensis* on control and experimental rats SOD, catalase, vitamin C and vitamin -E level

| Parameters | Control | <i>Camellia sinensis</i> (1g/kg) + | $CdCl_2$ | $CdCl_2$ + <i>Camellia sinensis</i> (250mg/kg) | $CdCl_2$ + <i>Camellia sinensis</i> (500mg/kg) | $CdCl_2$ + <i>Camellia sinensis</i> (1g/kg) |
|------------------|--------------------|------------------------------------|------------------|--|--|---|
| SOD | 14.02 \pm 0.98 | 14.59 \pm 0.68 | 7.34 \pm 0.56 | 8.92 \pm 0.98 | 12.99 \pm 0.86 | 13.16 \pm 1.01 |
| Catalase | 178.46 \pm 11.32 | 169.38 \pm 9.88 | 98.36 \pm 8.86 | 138.7 \pm 7.03 | 155.7 \pm 8.03 | 159.8 \pm 8.98 |
| Vitamin C | 1.91 \pm 0.07 | 1.89 \pm 0.05 | 1.38 \pm 0.12 | 1.47 \pm 0.11 | 1.58 \pm 0.63 | 1.65 \pm 0.59 |
| Vitamin E | 1.44 \pm 0.11 | 1.39 \pm 0.09 | 1.22 \pm 0.18 | 1.27 \pm 0.10 | 1.58 \pm 0.42 | 1.63 \pm 0.33 |

$CdCl_2$ - Cadmium chloride

Values are means \pm S.D for six rats.

Values not sharing a common superscript and differ significantly at $p < 0.05$ (DMRT)

Free radicals are chelating metal, ions inhibiting enzymatic systems responsible for free radical generation (Blaha et al., 2004; Dias et al., 2005). Superoxide anions are known to exert destructive effects on cellular components among that lipid peroxidation being one such consequence. The uptake, accumulation and toxicity of inorganic mercury in the kidney have been related to its binding to endogenous thiol-containing molecules (Zalups, 2000). Thiol-containing enzymes have been recognized as the targets of inorganic cadmium (Emanuelli et al., 1996; Nogueira, 2003). Moreover binding causes decreased glutathione levels, leading to increase in the levels of reactive oxygen species

(ROS), such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals (Stohs, 1995) which provoke lipid, protein and DNA oxidation (Clarkson, 1997).

Cadmium is known to increase oxidative stress by being a catalyst in the formation of reactive oxygen species, increasing lipid peroxidation, and depleting glutathione and protein-bound sulfhydryl groups. Cadmium also can stimulate the production of inflammatory cytokines and down regulates the protective function of nitric oxide formation (Navas-Acien et al. 2004).

Under normal condition, the redox state of the cell is largely linked to an iron (sometimes Cu) redox couple and is maintained within strict physiological limits. However, under stress conditions, an excess of $O_2^{\bullet-}$ releases "free iron" from iron-containing molecules catalyzes the breakdown of H_2O_2 (according to the Fenton reaction) and leads to the generation of the majority of the HO^{\bullet} (Platenik *et al.*, 2001).

Enhanced generation of ROS can overwhelm the intrinsic antioxidant defense in cells, and results in a condition known as 'oxidative stress'. Oxidative stress is associated with the production of ROS and is believed to be involved not only in the toxicity of xenobiotics but also in the pathophysiology of aging and several diseases including atherosclerosis, neoplastic diseases, diabetic complications, chronic inflammatory diseases of the gastrointestinal tract, diseases associated with cartilage, Alzheimer's disease, and other neurologic disorders (Stohs and Bagchi, 1995; Valko *et al.*, 2007).

Flavonoids are effective antidotes in acute and other metal poisoning of cadmium (2006a; Magdalan *et al.*, 2006b; Szelag *et al.*, 2003), exhibit potent antioxidant activity with free radical scavenging, metal chelation, antioxidant enzyme activation, reduction alpha-tocopherol radicals and oxidase inhibition (Fang and Yang, 2002). In recent years, much attention has been focused on the protective effect of naturally occurring antioxidants in biological systems against toxic heavy metals.

Vitamin-C and Vitamin E

Table 5: Explains that cadmium administered rats noticed decreased level of non-enzymatic antioxidants such as vitamin C and vitamin E significantly ($p > 0.05$) compared with the control rats, whereas, addition of *Camellia sinensis* (1g/kg) brought the deviated vitamin C, vitamin E levels towards normal level significantly, which were found to be closure to control rats.

Vitamin C is a primary preventive antioxidant in the cells and body fluids scavenges the free radicals and serves as a metabolic marker of toxicity (Pharikal *et al.*, 1988). The antioxidants, such as vitamin E (α-tocopherol) and vitamin C (ascorbic acid) are able to interact with oxidizing radicals directly (Jones *et al.*, 1995) also, terminates the chain reaction of lipid peroxidation in membranes and lipoproteins (Dieber-Rotheneder *et al.*, 1991). Whereas vitamin C scavenges aqueous-phase ROS by very rapid electron transfer and thus inhibits lipid peroxidation (Halliwell *et al.*, 1987), as well as reduces the oxidized tocopheroxyl radicals. Therefore, vitamin C and vitamin E function together to protect membrane lipids from damage (Frei, 1991).

The pharmacological effects are related to the anti-oxidant activity of flavonoids, arising through their ability to scavenge free radicals. When free radicals are generated in excess damages bio molecules, and therefore implicated in the etiology of several diseases and ageing (Ames *et al.*, 1993).

Previous studied have shown that tea catechins are excellent electron donors and effective scavengers of physiologically relevant reactive oxygen species *in vitro*, including superoxide anions (Velayudham., 2008), peroxy radicals, and singlet oxygen (Guo *et al.*, 1999). Most studies on the antioxidant effects of green tea are directly related to the total phenolic extracts, without considering the contributions of individual molecules, although various catechins, such as EGCG, ECg and EGC, have been linked to strong antioxidant activity in green tea extracts.

The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damages bacterial cell membrane (Hsu *et al.*, 2003). They also serve in plant defense mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and caused by microorganisms, insects, and herbivores (Akowuah and Ismail., 2005). The antibacterial activity of *Camellia sinensis* leaf against *Listeria monocytogenes* by disc diffusion method, the methanolic extract had greater antibacterial property as compared to the water extract (Mbata., 2008).

Methanolic extract of *Camellia sinensis* had greater antibacterial activity against *Staphylococcus aureus* and *Enterococcus* sp. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria (Souza *et al.*, 2005). In the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins, catecholic tannin plays the vital role in the plant defense mechanisms (Salvet *et al.*, 2001). The total phenolic content was found 0.7g/gram leaves extract.

It is well established that the non-enzymatic antioxidants such as vitamin C and vitamin E concomitantly decreased in heavy metal toxicity (Chia *et al.*, 2008; Chia *et al.*, 2008). The antioxidants, such as vitamin E (α-tocopherol) and vitamin C (ascorbic acid) are able to interact with oxidizing radicals directly (Jones *et al.*, 1995) also, terminates the chain reaction of lipid peroxidation in membranes and lipoproteins (Dieber-Rotheneder *et al.*, 1991). Whereas vitamin C scavenges aqueous-phase ROS by very rapid electron transfer and thus inhibits lipid peroxidation (Halliwell *et al.*, 1987), as well as reduces the oxidized tocopheroxyl radicals. Therefore, vitamin C and vitamin E function together to protect membrane lipids from damage (Frei, 1991).

Cadmium is one of the most toxic metal compounds released into the environment and is one of the important environment pollutant that can be ingested or inhaled from a variety of industrial and dietary sources. It is dangerous because humans consume both plants and animals that absorb cadmium efficiently and concentrate it within their tissues. Cadmium is a ubiquitous toxic metal that may induce oxidative damage by disturbing the pro-oxidant and antioxidant balance in the tissue. The mechanism of cadmium induced oxidative stress is not fully clarified. SOD, catalase and glutathione peroxidase (GPx) are enzymes that provide cellular protection against the damage caused by free radicals and ROS. Measurement of these enzyme activities is an indirect and non-invasive method that could be used to assess oxidant stress.

The pharmacological effects are related to the anti-oxidant activity of flavonoids, arising through their ability to scavenge free radicals. When free radicals are generated in excess damages bio molecules, and therefore implicated in the etiology of several diseases and ageing (Ames *et al.*, 1993).

Antioxidants in green and black tea-tea is brimming with antioxidants; the disease-fighting compounds that help your body stave off illness. Read the tea leaves, caffeine lovers. Tea is gaining ground over coffee. Even Starbucks is bucking up its tea menu. The health benefits of tea are one compelling reason: Green and black teas have 10 times the amount of antioxidants found in fruits and veggies. Studies of humans and animals show that the antioxidants in black and green teas are highly beneficial to our health, (Old John Weisburger, 2008).

Green tea, black tea, oolong tea -- they all come from the same tea plant, *Camellia sinensis*. The leaves are simply processed differently, explains Weisburger. Green tea leaves are not fermented; they are withered and steamed (Jeanie Lerche Davis, 2008).

All the tea from the camellia tea plants are rich in polyphenols, which are a type of antioxidant. These wonder nutrients scavenge for cell-damaging free radicals in the body and detoxify them. "Whether it's green or black, tea has about eight to 10 times the polyphenols found in fruits and vegetables." Black and green both have different types of antioxidants than fruits and vegetables. The arubigins, epicatechins, and catechins are among those listed in a USDA chart. All are considered flavonoids, a type of antioxidant. Brewed green and black teas have loads of those, the chart shows. (Herbal teas may also contain antioxidants but less is known about them, (Weisburger.)" both types of tea blocked DNA damage associated with tobacco and other toxic chemicals. The mechanism of detoxifying action of *Camellia sinensis* is most probably based on their high affinity

and ability to form insoluble complexes with mercury ions. This could be due to efficient chelating activity of *Camellia sinensis*. It was supported by (Salim *et al.*, 2013) findings proved that *Camellia sinensis* has potent anti-inflammatory and antioxidant capabilities.

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