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ORIGINAL RESEARCH ARTICLE

Evaluation of Radical Scavenging and Antioxidant Activities of Trahydrocurcumin: An *In vitro* and *In vivo* Study

Ramalingam Ramakrishnan and Leelavinothan Pari*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

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ABSTRACT

To examine the radical scavenging potentiality and antioxidant activity of Tetrahydrocurcumin (THC) in the *in vitro* free radical system and Cadmium (Cd) induced oxidative stress in rats. *In vitro* free radical scavenging activity of THC was assessed by using externally generated free radical such as 2, 2'-diphenyl- 1- picrylhydrazyl radical ($DPPH^{\bullet}$), 2, 2'- azinobis (3-ethylbenzo thiazoline- 6- sulfonic acid) radical ($ABTS^{+\bullet}$), superoxide and hydroxyl ions with ascorbic acid as standard. Effects of THC [Oral, 80 mg/kg body weight (b.w)] in Cadmium (Cd) induced oxidative stress (Oral, 5mg/kg b.w for 4 weeks) were assessed using male albino Wistar rats. Free radicals generated in different *in vitro* modals were remarkably controlled by THC. Elevated levels of lipid peroxidation end products and the decreased levels of antioxidants were observed in plasma of Cd intoxicated rats. Administration of THC followed by Cd significantly restored the above changes to near normal. This study suggests that the phytonutrient THC effectively controls the free radicals produced in the *in vitro* and Cd induced *in vivo* condition thereby exhibits its potent antioxidant activity.

Key words: Free radical, Tetrahydrocurcumin, Oxidative stress, Antioxidants and Cadmium toxicity.

Abbreviations*:* Cd, Cadmium; THC, Tetrahydrocurcumin; TBARS, thiobarbituric acid reactive substances; LOOH, lipid hydroperoxides; CD, conjugated dienes; GSH, reduced glutathione.

1. INTRODUCTION

Cadmium is one of the most dangerous heavy metal toxins widely distributed in the environment, exposed through occupational hazards and environmental pollution. Threat caused by Cd has increased exponentially in recent decades mainly because of urbanization and industrialisation as well as the multiple uses by humans [1,2]. Cd present in different materials discharged into the environment through polluted air (cigarette smoke, polluted air), water (industrial discharge) and soil (fertilisers, pesticides) reaches the system maximally by food chain [3,4]. After entering into the biological system, Cd disturbs the biochemical processes and leads to health abnormalities either directly or by protein bound metallothionein form [5]. Free radicals are the atoms or molecules containing one or more unpaired electrons in its outer most orbital and initiate the oxidation reactions. Highly reactive oxygen radicals; hydroxyl ($OH^{\bullet}$), superoxide anion radicals ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), nitrogen radicals; nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) and the carbon centred radicals; alkyl ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) are dominantly take part radical mediated oxidative deterioration processes [6,7]. Free radicals plays dual role in biological system, either beneficial or harmful to living organism according to their localisation and concentration. As for as concerning the heavy metal cadmium, this transition element does not able to generate the free radicals directly, instead by decreasing the available antioxidant in the system. Heavy metals like Cd inhibit the activity of vital antioxidant enzymes by substituting the metal cofactor in its active site [8] elevating the free radicals and the associated health problems in the intoxicated condition. Cadmium depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species

***Corresponding Author:** Leelavinothan Pari, **E-mail:** paaribalaji@gmail.com

such as superoxide ions, hydroxyl radicals and hydrogen peroxides in the biological system [9, 10]. These reactive oxygen species result in increased lipid peroxidation in cell membranes which may eventually leads to cell death. [11] Metal mediated free radical generation may cause various modifications to DNA bases, enhances the lipid peroxidation, and changes the Calcium and sulphydryl (SH) homeostasis, thereby aggravating the antioxidant defence system and causes several diseases including cancer, atherosclerosis diabetes and neurologic disorders [12, 13, 14] .

Synthetic chelating agents like dimercaptol, N-acetyl-DL-penicillamine, diethylene triamine pentaacetate, N-acetylcysteine, α-lipoic acid, 2, 3-dimercapto succinic acid and its derivatives are widely used as antidotes for heavy metal poisoning, but the side effect may worsen the host [14, 15, 16]. Natural medicines are much safer than synthetic drugs have gained popularity in recent years and lead to tremendous growth in phytopharmaceutical uses [17]. Studies proved that plants, herbs and phytochemicals have the property of ameliorating the metal toxicity in animal models [18]. Tetrahydrocurcumin is a colourless metabolite of curcumin, specifically used in fortified foods and cosmetic applications [19]. THC exhibits the antioxidant activity by controlling the free radical generation in animals [20]. The main objective of this study was to investigate the antioxidant activity of the Tetrahydrocurcumin (THC) using *in vitro* models and rats.

2. MATERIALS AND METHODS

2.1. Chemicals

Tetrahydrocurcumin (95.27% purity) was a gift sample provided by Sabinsa Corporation, USA. Cadmium chloride, 2-thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), reduced glutathione (GSH), ascorbic acid, vitamin E, 2, 2'-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$) and 2, 2'-azinobis - (3 - ethyl benzothiazoline – 6 - sulfonic acid) (ABTS) were obtained from Sigma chemical co., St. Louis, MO, USA. The rest of the chemicals utilized for this study were obtained from local firm (India) in analytical grade. Visible spectra measurements were done using spectrophotometer (ELICO 117).

2.2. *In vitro* antioxidant activity

Antioxidant and free radical scavenging activity of THC at different concentrations (5-50 µg/mL) were studied and compared using suitable standard ascorbic acid in different *in vitro* models. Assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The fifty percent of effective concentration (EC_{50}) was calculated from the graph of scavenging effect in percentage against the concentration of compounds.

2.2 .1 Free radical scavenging activity

The Free radical scavenging activity of the THC was measured in terms of hydrogen-donating or radical scavenging ability by the method of Mensor *et al*. [21] using $DPPH^{\bullet}$ as free radical generator. Briefly, 1.5 ml of $DPPH^{\bullet}$ solution (10^{-4} M, in 95% Ethanol) was incubated with 1.5 ml of THC at various concentrations (5 - 50 µg/mL). The reaction mixture was shaken well and incubated in dark condition. The control was prepared as above devoid of THC and the intensity of the existing purple colour was measured colorimetrically at 517 nm using ascorbic acid as standard. The radical scavenging activity (RSA) of THC was measured as a decrease in the absorbance of $DPPH^{\bullet}$ and was calculated as follows:

RSA (%) = (Control O.D - Sample O.D/ Control O.D) x 100

2.2.2 Total Antioxidant activity assay

Total antioxidant potential of THC was determined by the $ABTS^{\bullet}$ assay as described by Miller *et al*. [22] $ABTS^{+\bullet}$ cation radicals was produced by treating ABTS solution (7 mM) with ammonium persulfate (2.45 mM), allowed to stand in dark at room temperature for 12 -16 hrs. The reaction mixture containing 0.3 mL of $ABTS^{+\bullet}$ and different concentrations of THC (5 - 50 µg/mL) was made upto 3.5 mL using phosphate buffer (100 mM, pH 7.4). Intensity of the existing blue colour (0 min and 120 min) was measured at 734 nm using blank without THC and ascorbic acid as standard and the percentage inhibition was calculated. The total antioxidant activity (TAA) was calculated using the following equation:

TAA (%) = (Control O.D - Sample O.D/ Control O.D) x 100

2.2.3. Superoxide anion scavenging activity

Superoxide anion scavenging activity of THC was determined by the method of Nishmiki *et al*. [23] with slight modification. The reaction mixture containing 1 mL of NBT (100 µmol of NBT in 100mM phosphate buffer, pH 7.4), 1 mL of NADH (14.68 µmoL of NADH in 100 mM phosphate buffer, pH 7.4) and various concentrations of THC (5 - 50 µg/mL) were mixed thoroughly. The reaction was started by the

addition of 100 µL of PMS (60 µmol in 100 mM of phosphate buffer pH 7.4) then incubated at 30˚C for 15 min. The reaction mixture without THC was used as blank and the variations in colour intensity were measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity of THC.

2.2.4. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of THC was determined by the method of Halliwell *et al*. [24] The reaction mixture in a total volume of 1 mL containing 0.4 mL of 100 mM phosphate buffer with varying concentrations of THC (10 - 50 µg/mL), 0.2 mL of 500 mM ferric chloride, 0.1 mL of 1 mM ascorbic acid, 0.1mL of 10 mM H_2O_2 and 0.2 mL of 2 -deoxy ribose was taken. The contents were mixed thoroughly and incubated at room temperature for 60 min. After incubation, 1 mL of 1% TBA in 0.05 N NaOH and 1 mL of 28% TCA were added and the tubes were kept in a boiling water bath for 30 min with reagent blank containing buffer devoid of THC. Change in absorbance was read in a spectrophotometer at 532 nm and the percentage of scavenging activity was determined. Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity.

2.2.5. Reducing power

The reducing power of THC was determined according to the method of Oyaizu [25]. Varying concentrations of THC (5 - 50 µg/mL) in methanol were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrieyanide $[K_3Fe\ (CN)]_6$ and the mixture was incubated at 50^{o}C for 20 min. After incubation, 2.5 mL of TCA (10%) was added then centrifuged at 3000 rpm for 10 min. 2.5 mL of supernatant was mixed with equal volume of distilled water and 0.5 mL of 0.1% $FeCl_3$. Absorbance of the chromogen was measured at 700 nm using ascorbic acid as standard. Increased absorbance of the reaction mixture indicated increased reducing power.

2.3. Animals

Male albino Wistar rats, body weight of 180 – 200 g bred in Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the Institutional Ethical Committee (Vide. No. : 903, 2012) Annamalai University. The animals were fed on a pellet diet (Lipton India Ltd., Mumbai, India) and allowed to drink water *ad libitum*.

2.3.1 Experiment Protocol

Animals were randomized and divided into 4 groups of which each group containing 6 rats. The toxic dose of the Cd was selected based on the studies from Renugadevi and Prabu [26].

Group I: Control rats (Vehicle treated)

Group II: Rats were administered with THC (80 mg/kg b.w/day, orally) suspended in corn oil for 4 weeks.

Group III: Rats were administered with Cd as Cadmium chloride (5 mg/kg b.w/day, orally) in isotonic saline for 4 weeks.

Group IV: Rats were administered with Cd in saline (5 mg/kg b.w /day, orally) and THC in corn oil (80 mg/kg b.w /day, orally after 3 hrs of Cd administration) for 4 weeks.

2.3.2. Sample Preparation

At the end of the experimental study period, all the rats were anaesthetized by intramuscular injection of Ketamine hydrochloride (30 mg/kg b.w) and euthanized by cervical decapitation. Blood samples were collected in the tubes containing heparin as anticoagulant and the separated plasma (centrifugation at 3000 xg for 5 min) samples were used for various biochemical studies.

2.4. Biochemical studies

2.4.1. Estimation of lipid peroxidation products

Lipid peroxidation product TBARS were estimated colorimetrically by the method of Niehius and Samuelson [27]. In brief, 0.5 ml of plasma was treated with 2 ml of TBA-TCA-HCl reagent (0.37% Thiobarbituric Acid, 15% Trichloro Acetic acid and 0.25M Hydrochloric acid in 1:1:1 ratio), mixed thoroughly and incubated in a water bath for 15 min. Then, cooled and centrifuged. Absorbance of protein free filtrate was measured at 535 nm using reagent without plasma as blank and 1, 1', 3, 3' tetramethoxy propane as standard.

Lipid hydroperoxides (LOOH) in plasma was estimated by Jiang *et al*. [28]. 0.5 mL of plasma was treated with 0.9 ml of Fox reagent (88 mg of butylated hydroxy toluene (BHT), 7.6 mg of xylenol orange and 9.8 mg of ammonium iron sulphate were added to 90 ml of methanol and 10 ml of 250 mM H_2SO_4) and incubated at 37°C for 30 min. The intensity of the colour developed was read at 560 nm using reagent blank and H2O2 (0.2 M) as standard.

The levels of CD were assessed by the method of Rao and Racknagel [29]. In brief, 1.0 ml of plasma was mixed with 5.0 ml chloroform - methanol reagent (2:1v/v) and then centrifuged. To the supernatant, 1.5 ml of cyclohexane was added and the absorbance was read at 233 nm against a cyclohexane blank. The amount of CD formed was calculated using a molar extinction coefficient of 2.52×10^4 cm^{-1}.

2.4.2. Determination of non-enzymatic antioxidants

Concentration of ascorbic acid (vitamin C) in plasma was quantified by Omaye *et al.* [30]. In brief, to 0.5 mL of plasma, 1.5 mL of 6% TCA was added, mixed and centrifuged (3500 xg, 20 min). To 0.5 mL of protein free supernatant, 0.5 mL of DNPH reagent (2% DNPH and 4% thiourea in 9 N sulfuric acid) was added and incubated in room temperature for 3 h. After incubation, the reaction mixture was acidified by the addition of 2.5 mL of 85% sulfuric acid and the colour developed was measured colorimetrically at 530 nm.

Vitamin E present in the plasma was estimated by the method of Desai [31]. This fat soluble substance was extracted from plasma by treating 0.5 mL of plasma with 3.5 mL of lipid solvent (1.5 mL ethanol and 2.0 mL petroleum ether), mixed thoroughly and centrifuged. The supernatant was collected and evaporated to dryness. To the residue, 0.2 mL of 0.2% 2, 2 - dipyridyl and 0.2 mL of 0.5% ferric chloride was added and kept in dark for 5 min. then 4 mL of butanol was added. An intense red butanol layer obtained was read at 520 nm.

Reduced glutathione (GSH) content in the sample was estimated by Ellman method [32]. 0.5 ml of plasma was mixed with equal volume of 10% TCA and then centrifuged for 10 min at 5000 rpm. 0.5 mL supernatant was mixed with 2 ml of phosphate buffer (0.2 M, pH 8.0) and 0.5 ml of Ellman's reagent (19.8 mg % of 5, 5-dithiobisnitro benzoic acid in 0.1% sodium citrate), incubated at room temperature for 10 min and the absorbance was measured at 412 nm. A reagent blank containing the same without plasma and standards containing 20 -100 µg glutathione were processed similarly along with test samples.

2.5. Statistical analysis of the Data

Values are given as mean ±S.D for six rats in each group. The data for various biochemical parameters were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values not sharing a common letter differ significantly at $p<0.05$ [33].

3. RESULTS

***In vitro* antioxidant activity**

(**Fig 1-4**) shows the percentage radical scavenging activity and the antioxidant activity of THC and the standard ascorbic acid in different models like $DPPH^{\bullet}$ decolouration, $ABTS^{\bullet}$ assay, superoxide anion and hydroxyl radical scavenging activity methods. From the results it was inferred that the linear increase in free radical scavenging and the antioxidant property of THC and the ascorbic acid with the increase in concentration. The maximum activities observed in the highest concentration and the fifty percent of effective concentration (EC_{50}%) values of THC 33.20, 34.97, 32.83, 30.29 µg/mL and the ascorbic acid 6.57, 10.87, 16.25, 11.87 µg/mL respectively.

The ferric reducing power of the THC and standard ascorbic acid were shown in Fig.5. Reducing power of both the compound and the standard were increases with the increase in concentrations and expressing good linear relation values (THC: R^2=0.985, ascorbic acid: R^2=0.991).

***In vivo* antioxidant activity**

(**Table 1**) depicts the changes in the levels of lipid peroxidation products in plasma of control and experimental animals. In Cd treated rats, the levels of TBARS, LOOH and CD were significantly ($p < 0.05$) increased. Administration of THC (80 mg/kg b.w) followed by Cd restores these changes to near normal.

(**Table 2**) shows the changes in the levels of non - enzymatic antioxidants such as ascorbic acid, vitamin E and GSH in plasma of control and experimental rats. Significant ($p < 0.05$) decreases in the levels of non - enzymatic antioxidants were noticed in rats treated with Cd when compared to control rats. Treatment of THC reinstates the levels of non - enzymatic antioxidants in Cd intoxicated rats.

Table 1: Changes in the levels of lipid peroxidation markers in plasma of control and experimental rats

Parameters in Plasma	Control	Normal + THC (80 mg/kg)	Normal + Cd (5m /kg)	Cd (5 mg/kg) + THC (80 g/kg)
TBARS	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.23 ± 0.02^{b}	0.17 ± 0.01^{c}
LHP	10.80 ± 0.89^{a}	11.07 ± 0.70^{a}	14.72 ± 0.80^{b}	12.48 ± 1.06^{c}
CD	0.41 ± 0.03^{a}	0.43 ± 0.04^{a}	0.79 ± 0.06^{b}	0.52 ± 0.04^{c}

THC - Tetrahydrocurcumin; Cd - Cadmium. Values are mean ± SD for 6 rats in each group. The levels of TBARS in plasma were expressed as µmoles/dL and the levels of lipid hydroperoxides in plasma were expressed as m moles/dL. a-c In each row, means with different superscript letter differ significantly at $p<0.05$ (DMRT).

Table 2: Changes in the levels of non-enzymatic antioxidants in plasma of control and experimental rats

Parameters in Plasma	Control	Normal +THC (80 mg/kg)	Normal + Cd (5 mg/kg)	Cd (5 mg/kg) + THC (80 mg/kg)
Vitamin C	1.74 ± 0.20^{a}	1.76 ± 0.18^{a}	1.22 ± 0.12^{b}	1.49 ± 0.07^{c}
Vitamin E	1.24 ± 0.09^{a}	1.43 ± 0.10^{a}	0.79 ± 0.05^{b}	1.17 ± 0.20^{c}
GSH	20.20 ± 1.54^{a}	22.17 ± 1.69^{b}	14.78 ± 1.13^{c}	18.43 ± 1.06^{d}

THC-Tetrahydrocurcumin; Cd-Cadmium. Values are mean ± SD for 6 rats in each group. The levels of vitamin C, vitamin E and GSH as mg/dL. a-d in each row, means with different superscript letter differ significantly at $p<0.05$ (DMRT).

Fig 1: DPPH Free radical scavenging activity of Tetrahydrocurcumn and standard ascorbic acid

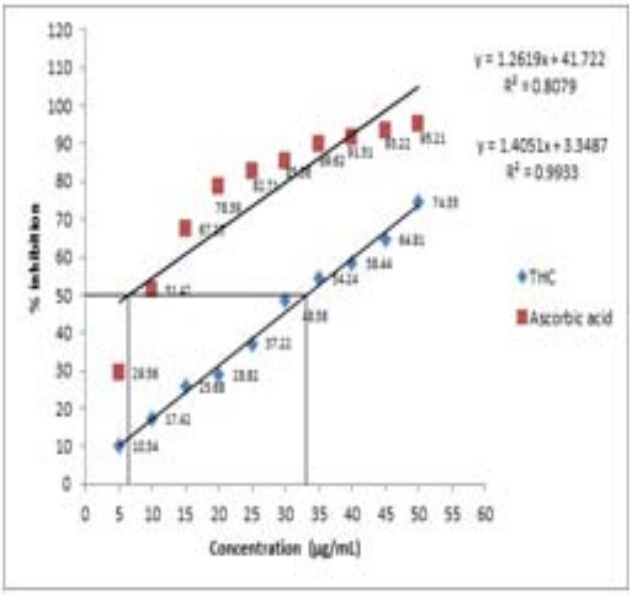


Fig 2: ABTS radical scavenging activity of Tetrahydrocurcumn and standard ascorbic acid

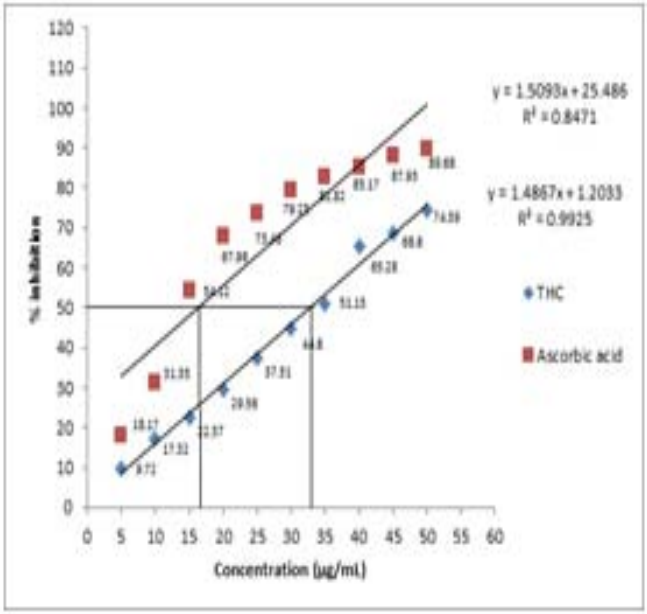


Fig: 3 Superoxide anion radical scavenging activity of Tetrahydrocurcumn and standard ascorbic acid

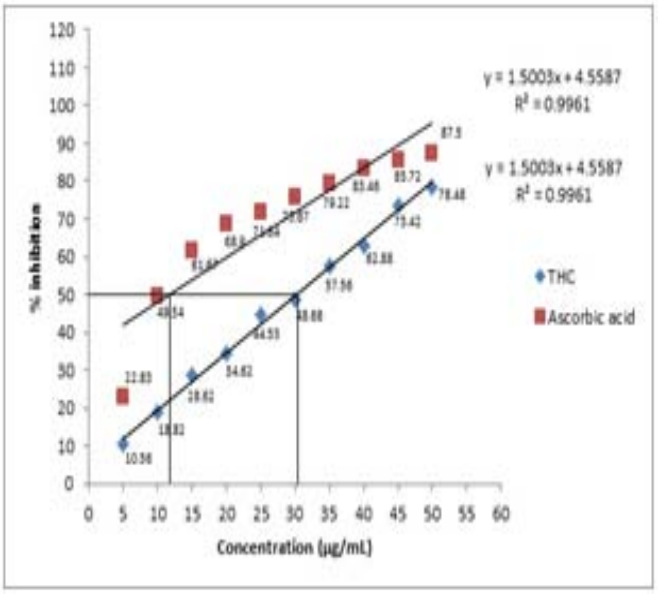


Fig: 4 Hydroxyl radical scavenging activity of Tetrahydrocurcumn and standard ascorbic acid

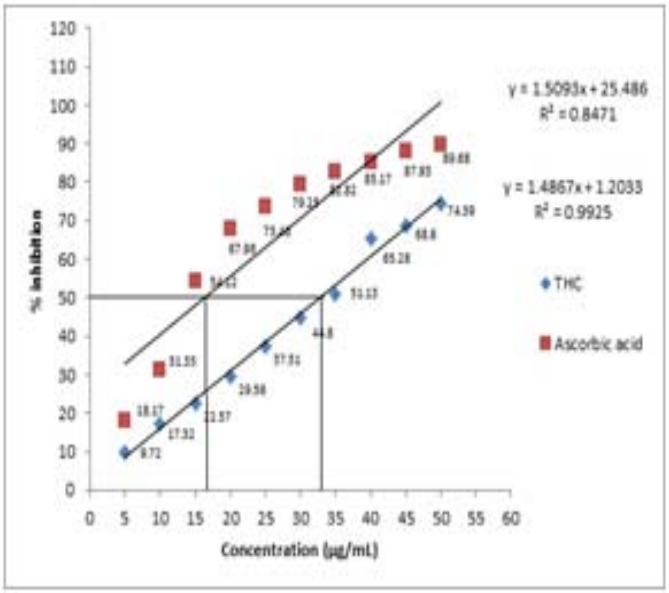
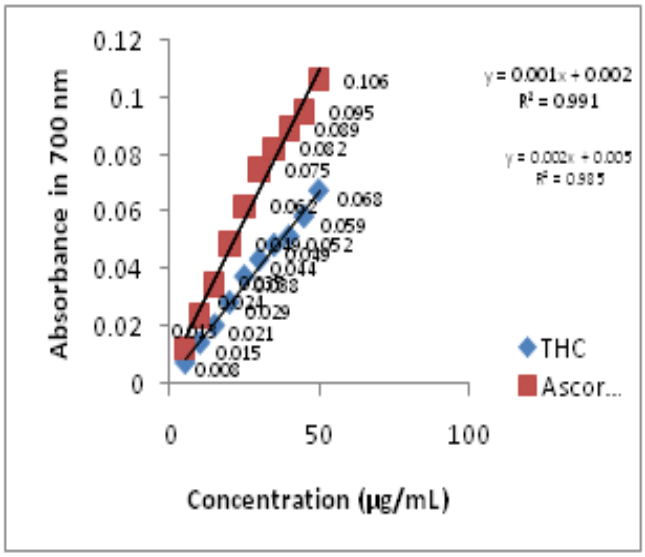


Fig 5: Ferric reducing power of THC and standard ascorbic acid



4. DISCUSSION

DPPH$^{\bullet}$ radical decolouration study was widely used to investigate the antioxidant activity of synthetic and phytocompounds. This stable free radical is a purple complex decolourised when scavenged by the antioxidants. Colour changed from purple to yellow after reduction by antioxidant was quantified by measuring the absorbance at 517 nm. The external reducing agents (antioxidants) donate electron, paired off with DPPH$^{\bullet}$ and decreases the colour intensity [34]. Hence, forth the increase in concentration of THC decreases the colour rapidly, moreover THC at 50µg/mL exhibits maximum radical scavenging activity (74.35% inhibition) among different concentrations. This might be due to the presence of diketone moiety in the of the heptane side chain of THC, which undergoes keto-enol tartomarism and donate electron to quench the DPPH$^{\bullet}$ radical.

ABTS$^{+\bullet}$ radical decolourisation study was an excellent method to assess the antioxidant property of compounds. Blue coloured ABTS radical generated in the presence of free radical inducer ammonium persulfate was quenched by the antioxidant and the extent of antioxidant character was quantified by measuring the existing colour intensity [35]. THC in the concentration of 50 µg/mL showed the maximum RSA (71.42). Decrease in the absorption at 734 nm proves the antioxidant role played by THC, which might be due to the electron donating phenolic group present in the aromatic ring.

Superoxide anions act as free radicals either directly or indirectly by forming H_2O_2, OH^- and singlet oxygen ($1/2O_2^{-\bullet}$) that have potential of reacting with biological macromolecules, thereby inducing tissue damage. Experimental study has confirmed that superoxide anions directly take part in initiation of lipid peroxidation.[36] Superoxide radical scavenging activity of THC was studied in terms of inhibition of superoxide radical generation. Auto oxidation of catecholamines generates the superoxide anions in the *in vitro* system. Superoxide radicals react with nitroblue tetrazolium (NBT) in the presence of NADH and produce blue colour formazan product. THC exhibits its activity by removing the available superoxide radicals responsible for the formation of dye. Highest RSA of 74.39% was observed at 50 µg/mL of THC. The intensity of colour is inversely proportional to the superoxide scavenging activity of THC, which might by the phenolic group present in the aromatic ring.

Hydroxyl radical scavenging activity was quantified by measuring the level malone dialdehyde which is an oxidation product of deoxyribose. This process was carried by the free radicals generated in the *in vitro* condition. Fenton's reaction mediated oxygen derived hydroxyl radicals in the presence of transition metal (Fe^{2+}) [36, 37] causes the degradation of deoxyribose into Malone dialdehyde which produces a pink chromogen with thiobarbituric acid. Intensity of the chromogen is inversely proportional to the scavenging activity of the antioxidant. The highest percentage scavenging activity of THC was observed at the concentration of 50 µg/mL and the percentage was 78.48%.

The antioxidant activity of the compound was assessed by its reducing potentiality. The reducing power of THC was determined by the reduction of Fe^{3+} to Fe^{2+} [38] which was measured at 700 nm. Increase in absorbance with increase in concentration expresses the reducing property of THC in the concentration dependent manner. Reducing power of THC might be with the quenching ability of the free radicals by donating electron and converting them to more stable products. Previous study confirmed that the enolic functional groups of the β-diketone moiety present in THC take response in its bioactivity [39].

Lipid peroxidation is the process of oxidative deterioration of lipids initiated by free radicals. Cadmium induces the free radical generation indirectly by displacing the iron and copper from various intracellular sites and increasing ionic concentration in the living system, which in turn causes the generation of free radicals through Fenton reaction [40]. Free radicals generated by

cadmium toxicity can attack the cell membrane, thus leading to disintegration and destabilization of the cell membrane [41]. Several studies have shown the elevated levels of lipid peroxidation end products in cadmium toxicity in rats [42, 43]. From our results, elevated levels of lipid decomposition products such as TBARS, LOOH and CD in plasma signify the cadmium induced the free radical mediated lipid peroxidation in rats. Oral administration of THC (80 µg/kg b.w) significantly reversed the changes to near normal.

The non - enzymatic antioxidants such as vitamin C, vitamin E and reduced glutathione (GSH) plays vital role in quenching the free radicals generated in Cd intoxicated rats. Vitamin C is a water soluble antioxidant (reducing agent) restrains the oxidation process and acts as antistress factor by controlling the oxidative stress in experimental animals.[44] It scavenges the free radicals produced by Cd induced lipid peroxidation.[45] Significant decrease in the level of vitamin C in plasma of Cd intoxicated rats could be due to the increased utilization of vitamin C as an antioxidant defence against oxidative stress. Vitamin E is a lipophylic chain breaking antioxidant and this membrane stabilizer plays a critical role in detoxication of Cd induced toxicity [46]. Depleted levels of vitamin E in plasma of Cd administered rats (5 mg/kg b.w) signify that it may take part in sequestering the released free radicals. GSH, the free sulfydril group containing tripeptide functions as free radical scavenger and protects the cellular system against the noxious effects caused by lipid peroxidation [47]. In our study, Cd intoxicated rats exhibited a significant decrease in the level of GSH in plasma of Cd administered rats. This might be due to the increased utilization for scavenging free radicals directly by regenerating the oxidised vitamin C and vitamin E or by acting as the substrate in enzyme catalysed antioxidant systems (Glutathione peroxidise and glutathione transferase). These three antioxidants functions in a coordinated manner to curb the free radical mediated injury [48, 49]. Administration of THC followed by Cd significantly restored the changes to near normal in the *in vivo* rat model, this might be due to the free radical trapping keto group in the side chain in its skeleton.

5. CONCLUSION

From the above results, it can be concluded that THC exhibits potent free radical scavenging and strong antioxidant activity, which was confirmed by the different *in vitro* radical scavenging studies and the plasma antioxidants rats. This combined *in vitro and in vivo* research may strengthen the versatile use of phytonutrients like tetrahydrocurcumin in therapeutic applications.

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