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ANTIOXIDANT PROPERTIES OF THE EXTRACT OF WHOLE PLANT PARTS OF *COLDENIA PROCUMBENS*

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ABSTRACT

Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality. Out of all the cancers colon cancer is one of the most common cancers in the world. Major causes of cancer are oxidative stress. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compare with normal cells, the redox imbalance thus may be related to oncogenic stimulation. Lots of anticancer drugs are in the market, but the main problem associated with these drugs is their side effects. Because of chemotherapy treatment side effects, the patient needs secondary palliative care treatment. Plant medicines are well known for their non-toxic side effects, so the objective of the study is to develop a drug from medicinal plant against colon cancer with non-toxic side effects. In the present study, C*oldenia procumbens* was used to study the Antioxidant potential of the extract and to synthesize new anticancer moiety. DCP was also studied for its *in vitro* antioxidant property by different methods. DCP has a hydrogen donating property, which may be attribute to the good antioxidant activity.

Key words: C*oldenia procumbens,* Antioxidant, *invitro, invivo*, free radicals.

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INTRODUCTION

Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality. Out of all the cancers colon cancer is one of the most common cancers in the world. Every year 1.2 million patients are diagnosed for colon cancer. The rate of colon cancer incidence was low in India but is presently increasing; out of 3.5 million cancer cases, 35,000 suffer from colon cancer. Major causes of cancer is oxidative stress. Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compare with normal cells, the redox imbalance thus may be related to oncogenic stimulation.

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Permanent modification of genetic material resulting from oxidative damage incident represents the first step involved in mutagenesis, carcinogenesis and aging. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been noted in various tumors, strongly implicating such damage in the etiology of cancer. ROS-induced DNA damage involves single-strand or double-strand DNA breaks, purine, pyrimidine, or deoxyribose modification and DNA cross-links. DNA damage can result in arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis. The most extensively studied DNA lesion is the formation of 8-Hydroxyguanine (8-OH-G). This lesion is important because it is relatively easily formed and is mutagenic and therefore is a potential biomarker of carcinogenesis. DNA damage, mutations, and altered gene expression are thus all key players in the process of carcinogenesis. The

involvement of oxidants appears to be the common denominator to all these events.

In addition to ROS, reactive nitrogen specie (RNS), such as peroxynitrates and nitrogen oxides have also been implicated in DNA damage. Upon reaction with guanine, peroxynitrate has been shown to form 8-nitroguanine.due to its structure, this adduct has the potential to induce G: C-> T: A transversion. While the stability of this lesions in DNA is low, in RNA, however, this nitrogen adduct is stable. The potential connection between 8-nitroguanine and process of carcinogenesis is unknown.

Other than extensive studies devoted to the role of oxidative nuclear DNA damage in neoplasia, there exists evidence about the involvement of mitochondrial oxidative DNA damage in the carcinogenesis process. Mutations and altered expression in mitochondrial genes encoding for complexes I, II, III, IV and V, and in the hyper variable regions of mitochondrial DNA, have been identified in various human cancers. Hydrogen peroxides and other reactive oxygen species have been implicated in the activation of nuclear genes that are involved in mitochondrial biogenesis, transcription, and replication of the mitochondrial genome. Although the region of tumour cells that process mutated mitochondrial DNA and the extent to which mitochondrial DNA alterations participate in the cancer process have not been satisfactorily established a significant amount of information supporting the involvement of mitochondria in carcinogenesis exists. This connection supports the fact the fragments of mitochondrial DNA have been found to be inserted in to nuclear DNA, suggesting a possible mechanism for activation of oncogenesis. Apart from DNA damage, the lipid peroxidation process has been implicated in the mechanism of carcinogenesis. Once formed, lipoperoxyl radicals ($ROO^{.}$) can be rearranged via a cyclization reaction to endoperoxides with the final product of the peroxidation process being Malondialdehyde (MDA). The major aldehyde product of lipid peroxidation other than Malondialdehyde is 4-hydroxynonenal (HNE). MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. NHE has powerful effects on signal transduction pathways which in turn have a major effect on the phenotypic characteristic of cells. MDA can react with DNA bases G, A, and C; to form adducts M1G, M1A, and M1C respectively

The role of oxidative stress in development of colorectal cancer was studied by several workers. Chronic inflammation in the gastrointestinal tract increases the risk for the development of cancer by an incompletely understood pathway, which may involve microsatellite instability (MSI). Low frequency of MSI referred as "MSI-L" occurs frequently in chronically inflamed non-neoplastic tissue. It is proven that oxidative stress is a potential mutagen leading to accumulation of frame shift mutations and may contribute to MSI in the setting of chronic inflammation. It has been found that the cells obtained from the neoplastic tissue presented oxidative DNA damage greater than in the cells from normal tissue. The cells isolated from the neoplastic mucosal tissue of the colon presented significantly greater mean extent of DNA strand breakage than the cells isolated from normal tissue.

Lots of anticancer drugs are in the market, but the main problem associated with these drugs is their side effects. Because of chemotherapy treatment side effects, the patient needs secondary palliative care treatment. Plant medicines are well known for their non-toxic side effects, so the objective of the study is to develop a drug from medicinal plant against colon cancer with non-toxic side effects. Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes and saponins and it is found to contain a variety of triterpenes and steroidal compounds. And also to find out, a newer synthetic drug, for its anti colon cancer potential and its toxic profile. In the present study, C*oldenia procumbens* was used to study the Antioxidant potential of the extract and to synthesize new anticancer moiety.

It is a prostrate herb usually lying quite flat on the ground, common on dry rice grounds, stem reaching 10 -50 cm long, shaggy with white hairs, branches often numerous. It is distributed in tropical and subtropical zones and found widely in South India. Leaves are crisped, alternate, short and sessaile, rounded at the apex, warty hair with rosette of basal cells, veins 4-6 pairs on each side. The leaves are use as poultice to mature abscesses and applied to rheumatic swellings. The expressed oil is reputedly used as a liniment applied to swollen knees and joints

MATERIALS AND METHODS

Plant material

Whole plant parts of C*oldenia procumbens* were collected from local region and District of Tiruvelveli, Tamilnadu, India in the month of April 2012. The botanical identity was confirmed and authenticated by a Taxonomist Dr. V.Chelladurai (Research Officer, Botany, (C.C.R.A.S) Government of India. The plant was dried under controlled temperature, powdered and passed through 40-mesh sieve.

150g of powdered plant material was packed in Soxhlet apparatus and refluxed with Dichloroethane until to get a clear solution. The extract was dried and weighed amount of the dried DCP was suspended in Distilled water and was used for the present study.

IN-VITRO ANTI OXIDANT STUDY

Scavenging of Superoxide radical (Malaya Gupta *et al*., 2004)

Super oxide dismutase scavenging activity was performed with DMO and at absorbance 560nm.

% scavenging activity was calculated by using the

formula

%scavenging = (Abs $_{control}$- Abs $_{sample}$/Abs $_{control}$) X 100

Scavenging of hydrogen peroxide radicals (Arulmozhi S *et al*., 2010)

Preparation of test and standard solutions.

30mg of the two plant extracts and the standard (ascorbic acid) was accurately weighed and separately dissolved in 10ml of methanol. These solutions were serially diluted with methanol to obtain to obtain the lower concentrations ranging from 50-250 μg/ml. The activity was determined using hydrogen peroxide and tested at 230nm using formula.

%scavenging = (Abs $_{control}$- Abs $_{sample}$/Abs $_{control}$) X 100

Nitric Oxide Scavenging Activity (Pooja *et al*., 2010)

Preparation of test & standard solutions

Different concentrations from 500 to 2500 microg/ml of the two plant extracts and as well as ascorbic acid (standard) were prepared in methanol and was mixed with phosphate buffer (2.5ml) and Sodium nitroprusside (2.5ml). Mixture is incubated at 25°c for 30 minutes. A portion of Griess reagent (1.5ml) was added to 1.5 ml of the reaction mixture. The absorbance was measured at 546nm. Increased absorbance of the reaction mixture indicated the increased reducing power scavenging activity was calculated by using the formula

%scavenging = (Abs $_{control}$- Abs $_{sample}$/Abs $_{control}$) X 100

β-Carotene Linoleate Model (Patel Rajesh M, 2011)

Prepation of test & standard solutions

Different concentrations 10-200mg/ml of the two plant extracts and ascorbic acid (standard) prepared in methanol. A solution of β-carotene is prepared by dissolving 2mg of β-carotene in the 10ml of chloroform. This solution is pipette (2ml) into a 100ml round bottomed flask. After removal of chloroform under vacuum, 40mg of purified linoleic acid, 400mg of Tween 40 emulsifier and 100ml of aerated distilled water was added to the flask with vigorous shaking. Aliquot (4.8ml) of this emulsion was transferred into test tubes containing different concentrations of the the two extracts (0.2ml). As soon as the emulsion was added to each tube, the zero time absorbance is measured at the 470nm using a spectrophotometer. The tubes were placed at 50°c in a water bath, and measurement of absorbance is recorded after 2hrs. A blank devoid of β-carotene, is prepared for background substraction. The same procedure was separated with the ascorbic acid, as a positive control. Antioxidant activity was calculated using the following equation.

Antioxidant activity =

$$\frac{\beta\text{-carotene content after 2hr of assay} \times 100}{\text{Initial } \beta \text{ carotene content}}$$

DPPH RADICAL SCAVENGING ASSAY (Patel Rajesh M, 2011)

0.3mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 1ml of various concentrations of sample and the reference compound (10, 20, 30, 40 and 50 μg/ml), were shaken vigorously and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Antiradical activity was expressed as inhibition percentage (I %) and calculated using the following equation:

Inhibition percentage (I %) =

(Abs $_{control}$- Abs $_{sample}$/Abs $_{control}$) X 100

In the previous equation the term Abs $_{sample}$ was substituted with (Abs $_{sample}$-Abs $_{blank}$). Different sample concentrations were used in order to obtain calibration curves and to calculate the EC50 values (EC50: concentration required to obtain a 50% radical scavenging activity).

ABTS Radical Cation Decolorization Assay (Pellegrini N *et al.,* 1991)

Prior to assay, $ABTS^{\bullet+}$solution was diluted in methanol and absorbance was adjusted to 0.70 (±0.02) at 734 nm. An aliquot of each sample of 50 μl was mixed with 950 μl of diluted ABTS cation radical solution. The absorbance was measured at 734 nm using an UV spectrophotometer after the solution has been allowed to stand for 6 min at room temperature. A control reaction was carried out without the test sample. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using following equation and IC_{50} are also calculated.

Inhibition percentage (I %) =

(Abs $_{control}$- Abs $_{sample}$/Abs $_{control}$) X 100

Ferric Reducing Ability Of Plasma/Ferric Reducing Antioxidant Power (FRAP) (Benzie IFF & Strain JJ, 1996; Benzie IFF & Strain JJ, 1999)

To 900 μl of FRAP reagent added different concentrations of sample solution (10, 20, 30, 40 and 50 μg/ml) and the final volume was made up to 1ml. The increase in absorbance at 593 nm was measured at 4 min. $FeSO_4.7H_2O$ was used as a standard. FRAP value was expressed as mmoles/100 on dry weight basis using the calibration curve of Fe^{2+}.

***Invivo* activity**

Selection and acclimatization of animals

Male Wistar rats were purchased from the National Institute of Nutrition, Hyderabad. Rats having

age of 5 weeks and 150gm body weight were used for the study. All the rats were kept at room temperature of 22 ± 2^0 C under 12 hr dark-light cycles, humidity was maintained at60-70% in the animal house. Rats were fed with modified pellet diet, and water *ad libitum* freely throughout the study (including 1 week for acclimatization). All animal procedures were performed in accordance with the recommendations for CPCSEA the proper care and use of laboratory animals. The proposal of the present study was approved by IAEC of RVS College of Pharmaceutical Sciences, Coimbatore. (IAEC NO: IAEC/ 1012/ C/ 06/ CPCSEA approved on 24.12.2010)

Treatment schedule

After the administration of DMH, the animals were grouped into three groups of six animals in each. One group of animals were treated as control received normal saline only, out of three, two groups received 200 & 400mg/kg dose of DCP for 30 weeks. During the course of study individual animal body weight was recorded, weekly. And % difference in the weights between the groups was calculated.

Animal Experimental Design

Group 1	Normal saline
Group 2	DMH only (weekly once)
Group 3	DMH (weekly once) + DCP extract (200mg/kg dose), PO daily
Group 4	DMH (weekly once) + DCP extract (400mg/kg dose). PO daily

Preparation of drug samples

The two plant extracts were weighed at a dose of 200mg/kg and 400mg/kg and dissolved in distilled water to provide a clear solution, which was administered to the animals through oral route.

Enzymatic antioxidants assays (Lowry OH *et al.,* 1951)

Estimation of Proteins

Protein was estimated by the method of Lowry *et al.* (1951).

Superoxide dismutase [SOD, EC 1.15.1.1]

SOD was assayed by the method of Kakkar *et al*. (1984).

Procedure

0.5 ml of the tissue homogenate (homogenized in 0.052 M sodium pyrophosphate buffer pH 8.3) or 0.5 ml erythrocyte lysate was diluted to 1.0 ml with ice-cold water followed by the addition of 2.5 ml ethanol and 1.5 ml chloroform (chilled reagents), shaken for 90 sec at 4ºC and then centrifuged. The enzyme activity in the supernatant was determined as follows: The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml PMS, 0.3 ml NBT and appropriately diluted enzyme preparation in a total volume of 3.0 ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at 30ºC for 90 sec, the reaction was stopped by the addition of 1.0 ml glacial acetic acid. The reaction mixture was stirred vigorously, shaken with 4.0 ml n-butanol and was allowed to stand for 10 min. After centrifugation the colour intensity of the chromogen in butanol layer was measured in a colorimeter at 520 nm. A system devoid of enzyme served as control.

The enzyme concentration required to produce 50% inhibition of chromogen formation in one minute under standard condition was taken as one unit. The specific activity of the enzyme is expressed as enzyme required for 50% inhibition of NBT reduction/min/mg Hb for erythrocyte lysate and enzyme required for 50% inhibition of NBT reduction/min/mg protein for tissues.
The level of SOD was expressed as units/min/mg protein.

Catalase [CAT, EC 1.11.1.6]

The activity of CAT was determined in erythrocyte lysate and tissue homogenate by the method of Sinha (1972).

Procedure

Tissue homogenate was prepared in phosphate buffer. To 0.9 ml of phosphate buffer, 0.1 ml erythrocyte lysate or tissue homogenate and 0.4 ml H_2O_2 were added. The reaction was arrested after 15, 30, 45 and 60 sec by adding 2.0 ml dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the colour developed was read at 590 nm. Standards in the concentration range of 20-100 ump were processed as for test. The specific activity of the enzyme is expressed as µmoles of H_2O_2 utilized/min/mg Hb for erythrocyte lysate and µmoles of H_2O_2 utilized/min/mg protein for tissues.

Glutathione peroxidase [GPx, EC 1.11.1.9]

The activity of GPx was assayed in erythrocyte lysate and tissue homogenate by the method of Rotruck *et al*. (1973).

Procedure

0.2 ml of Tris-HCl buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.2 ml enzyme preparation (erythrocyte lysate or tissue homogenate) were added and mixed well. To this, 0.2 ml GSH followed by 0.1 ml H_2O_2 were added. The contents were mixed and incubated at 37ºC for 10 min. The reaction was arrested by the addition of 0.5 ml TCA. The tubes were centrifuged and the remaining GSH was determined colorimetrically at 340 nm.

The activities are expressed as µmoles of GSH utilized/min/mg Hb for erythrocyte lysate and µmoles of GSH utilized/min/mg protein for tissues.

Reduced glutathione (GSH)

GSH in erythrocyte lysate and tissues were estimated by the method of Ellman (1959). 1.0 ml of erythrocyte lysate or tissue homogenate was precipitated with 2.0 ml TCA and centrifuged. To 1.0 ml of the supernatant, 3.0 ml phosphate buffer and 0.5 ml Ellman's reagent were added. The yellow colour developed was read in a colorimeter at 412 nm. A series of standards (20-100g) were treated in a similar manner along with a blank containing 1.0 ml buffer.

The amount of GSH is expressed as µg/dl erythrocyte lysate and mmoles/mg tissue.

To this 0.1ml of the homogenate, 0.9ml of water, 4.5ml of alkaline coppersulphate reagent were added and allowed to stand in the room temperature for 10 minutes. To this 0.5ml of Folin'reagent was added. After 20 minutes, the color developed was measured at 640nm. The level of protein present was expressed as mg/g tissue or mg/dl.

Estimation of gluconeogenic enzymes

Isolation of mitochondria and microsomes

The mitochondria of liver and kidney were isolated by the method of Johnson and Lardy (1967).

A 10% (w/v) homogenate was prepared in 0.05 M Tris-HCl buffer, pH 7.4 containing 0.25 M sucrose and centrifuged at 600 x g for 10 min. The supernatant fraction was decanted and centrifuged at 15,000 x g for 5 min. The resultant mitochondrial pellet was then washed and resuspended in the same buffer.

The post mitochondrial fraction was further centrifuged at 105,000 x g for 60 min. The microsomal pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.5 containing 0.15 M KCl. The purity of mitochondrial and microsomal fractions was assessed by measuring the activities of succinate dehydrogenase and glucose-6-phosphate dehydrogenase respectively.

Frutose-1, 6-diphosphatase (Fructose-1, 6-diphosphatase Phospohydrolase)

Fructose-1, 6-diphosphatase **was** assayed by an adaption of the procedure described by Gancedo and Gancedo (1971). The assay medium in a final volume of 2ml contained 1.2Ml of buffer, 0.1 ml of substrate solution, 0.23ml of MgCL2, 0.1 ml of KCL solution, 0.25 ml of EDTA solution and 0.1 ml of enzyme. The incubation was carried out at 37^0 C for 15 min. The reaction was terminated by the addition of 1 ml of 10% TCA solution. The suspension was centrifuged and the phosphorous content of the supernant was estimated by the method of Fiske and subbarow *et al.* (1925). Protein was determined by the method of Lowry *et al.,* (1951). The enzyme activity is expressed as nmoles of Pi liberated/min/mg protein.

Glucose-6-phosphatase(Glucose-6-phosphatephospohydrolase)

Glucose-6-phosphatese was assayed according to the method of King (1965). The incubation mixture in a total volume of 1 ml contained 0.3 ml of buffer, 0.5 ml of substrate , and 0.2 ml of enzyme solution .Incubation was carried out at 37^0 C for 60 min, The reaction was terminated by the addition of 1 ml of 10% TCA solution. The suspension was centrifuged and the phosphorus content in the supernatant was estimated.

The enzyme activity is expressed as nmoles of Pi liberated/min/mg protein.

Lipid Peroxidation Assay[19]

Added 140 µL of Standards or Samples to a microcentrifuge tube. Added 455 µL of diluted Reagent R1 to each tube and vortex. For MDA Only: Added 105 µL 37% HCl (12 N HCl) to each tube and mixed well. For MDA + DCP: Added 105 µL Reagent R2 (MSA) to each tube and mixed well. Incubated at 45°C for 60 minutes. Centrifuged samples at 15,000 x g for 10 minutes to obtain a clear supernatant. Transferred the 3 x 150 µL of the supernatant to the microplate and read at 586 nm. I for a sample plate layout. The color was stable for at least an hour at room temperature, or 2 hours at 4°C when stored in the dark dark (Devasagayam TPA & Boloor KK, 2003).

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RESULTS:

Invitro Antioxidant Activity

Scavenging of superoxide radical by alkaline of DCP results shown in table 1. and figure 1. In this DCP extract Scavenging activities were more comparable to that of standard. The standard showed 85.77, while DCP 97.64. Scavenging of hydrogen peroxide radical of DCP results shown in table 2 and figure 1. In this DCP extract Scavenging activities more compare to that of standard. The standard showed 79.08, while DCP 91.87. Nitric oxide scavenging activity results of DCP shown in table 3. and figure 1. In this DCP extracts scavenging activities are less compare to that of standard. The standard showed 78.50 while DCP 30.65. Scavenging of Linoleic Acid Radical By B-Carotene Linoleate Model results of DCP shown in table 4 and figure 1. In this DCP extract scavenging activities are less compare to that of standard. The standard showed 69.01 while DCP 50.80. Scavenging activity of DPPH Radical results of DCP shown in table 5. and figure 1. In this DCP extract the scavenging activities are low compare to that of standard. The standard showed 84.32 ± 0.376 while DCP 72.42 ± 0.376.

Scavenging activity of ABTS Radical results of DCP shown in table 6. and figure 1. In this DCP extract the scavenging activities are more compare to that of standard. The standard showed 83.44 while DCP 71.20. Inhibitory Activity of DCP results are shown in table 7.

and figure 1. In this DCP extract the scavenging activities are more compare to that of standard. The standard showed 90.48 while DCP 96.44.

In-Vivo Anti Oxidant Activity in Colon Tissue for DCP results were shown in Table 8, and figure 2. In this DCP extract showed significant anti Oxidant activity than standard drug. *In-Vivo* Anti Oxidant Activity in Liver Tissue for DCP results were shown in Table 9 and figure 3. In this DCP extract showed significant anti Oxidant activity than standard drug.

Estimation of Fructose 1 – 6 Diphosphatase and Estimation of Glucose – 6 Phosphate for DCP results were shown in Table 10 and figure 4. In this DCP extract showed significance than Standard. In this DCP extract showed more significant reversal than DMH treated group.

In DMSO model, scavenging of Superoxide radical by the DCP was comparable with the Ascorbic acid, showed the antioxidant potential of DCP. % scavenging activity of DCP was found to be 96.36at 200 µg/ml when compared with Ascorbic acid by 80.74 at 200 µg/ml.

Vitamin C is an important antioxidants, it significantly decreases the adverse effects of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases by scavenging/ neutralizing them.

Hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (•OH), resulting in initiation and propagation of lipid peroxidation. Ascorbic acid the antioxidant vitamin, significantly decreases the adverse effects of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules. DCP significantly scavenged the hydrogen peroxide radical when compared to the Ascorbic acid showed the antioxidant potential of DCP. The % scavenging activity of DCP was found to be 91.78 at 250 µg/ml when compared to ascorbic acid showed 74.01 only at 250 µg/ml

Superoxide dismutase can catalyze dismutation of O2 •− into H_2O_2, which is then deactivated to H_2O by Catalase or glutathione peroxidase. Normally, superoxide dismutase works in parallel with selenium dependent glutathione peroxidase, which plays an important role in the reduction of hydrogen peroxide in the presence of reduced glutathione (GSH) forming oxidized glutathione (GSSG), and thus, it protects cell proteins and cell membranes against oxidative stress. Glutathione peroxidase has a key role in enzymatic defence systems and reduces organic peroxides.Vitamin C is an important antioxidants, it significantly decreases the adverse effects of reactive species such as reactive nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases by scavenging/ neutralizing them.

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.

In scavenging of Nitric oxide radical, DCP non significantly scavenged the nitric oxide radical when compared with the Ascorbic acid scavenging effect. % scavenging activity of DCP was found to be 35.71 at 2500 µg/ml when compared with Ascorbic acid by 78.57at 2500 µg/ml. The linoleic acid free radical attacks the highly unsaturated b-carotene models. The presence of different antioxidants can hinder the extent of b-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

DCP significantly scavenged the β-Carotene Linoleate radical when compared to the Ascorbic acid showed the antioxidant potential of DCP. DCP showed the % scavenging activity by 96.29 at 2000 µg/ml when compared to ascorbic acid showed 59.15 only at 2000 µg/ml. Electrons become paired off and the solution loses color stochiometically depending on the number of electrons taken up. illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of the extract and the standard quercetin as a reference compound. The radical scavenging activity of DCP was evident at all the concentrations but only at moderate level not as significant as that of standard quercetin. The scavenging activity of the DCP was increased with increase in concentration of extract and that of the standard. DCP showed the % scavenging activity by 72.43 at 400 µg/ml when compared to quercetin showed 88.44 at 400 µg/ml.

DCP significantly scavenged the DPPH radical. The 1, 1-diphenyl -2-picryl hydroxyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as extract of plants in a relatively short time. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow (diphenyl picryl hydrazine) after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. In the present study DCP showed significant scavenging of DPPH radical when compared with standard compound.

The ABTS radical cation method described gives a measure of the antioxidant activity of the range of carotenoids, phenolics, and some plasma antioxidants, determined by the decolorization of the ABTS•1, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. ABTS•1 decolorization assay compared with the myoglobin/ABTS assay monitored at 6 min. The latter assay involved

continuous formation of the ABTS radical cation from ferryl myoglobin, derived from met-myoglobin and hydrogen peroxide in the presence of the reductants.

DCP significantly scavenged the ABTS radical cation, when compared to the STD. the anti oxidant potential of DCP. DCP shows the % scavenging activity by 71.2 at 100 µg/ml when compared to ascorbic acid showed 83.44only at 100 µg/ml. the antioxidant property were expressed as concentration dependent.

Table 1: Scavenging of superoxide radical by alkaline DMSO

S.No	Concentration (ug/ml)	Absorbance	% scavenging activity
	Control (Blank)	2.119	
Ascorbic acid			
1	50	1.839	13.21
2	100	1.697	19.90
3	150	0.484	76.88
4	200	0.404	78.84
5	250	0.258	85.77
DCP			
1	50	1.654	21.94
2	100	0.640	69.79
3	150	0.460	78.29
4	200	0.078	96.31
5	250	0.050	97.64

Table 2: Scavenging of hydrogen peroxide radial

S.No	Concentration (ug/ml)	Absorbance	% scavenging activity
	Control (Blank)	2.032	
Ascorbic acid			
1	50	1.735	14.61
2	100	0.804	60.43
3	150	0.594	70.76
4	200	0.461	77.31
5	250	0.425	79.08
DCP			
1	50	0.807	60.28
2	100	0.154	92.21
3	150	0.174	91.43
4	200	0.144	92.91
5	250	0.165	91.87

Table 3: Nitric Oxide Scavenging Activity

S.No	Concentration (ug/ml)	Absorbance	% scavenging activity
	Control (Blank)	0.014	
Ascorbic acid			
1	50	0.007	50.00
2	100	0.006	64.28
3	150	0.005	64.25
4	200	0.005	64.20
5	250	0.003	78.50
DCP			
1	50	0.007	50.00
2	100	0.007	49.06
3	150	0.009	35.70
4	200	0.018	30.70
5	250	0.019	30.65

Table 4: Scavenging Of Linoleic Acid Radical by β -Carotene Linoleate Model

S. No	Concentration (ug/ml)	Initial absorbance	Absorbance after 2hrs	% scavenging activity
Ascorbic acid (STD)				
1	500	0.062	0.036	63.31
2	1000	0.068	0.043	64.28
3	1500	0.072	0.040	56.70
4	2000	0.071	0.042	59.15
5	2500	0.070	0.045	69.01
DCP				
1	500	0.029	0.025	89.64
2	1000	0.028	0.024	92.81
3	1500	0.025	0.026	96.40
4	2000	0.027	0.025	96.20
5	2500	0.030	0.020	55.80

Table 5: Scavenging Activity of DPPH Radical

S. No	Concentration (μg/ml)	% scavenging activity
Std (quercetin)		
1	50	73.24±0.015
2	100	76.23±0.022
3	200	80.44±0.01
4	300	83.60±0.02
5	400	84.42±0.024
DCP		
1	50	45.65±0.033
2	100	54.95±0.760
3	200	62.30±0.315
4	300	66.53±0.051
5	400	72.42±0.376

Table 6: ABTS Radical Cation Assay

S. No	Concentration (μg/ml)	% scavenging activity
Std (quercetin		
1	20	31.23
2	40	42.60
3	60	64.10
4	80	75.10
5	100	83.44
DCP		
1	20	18.76
2	40	27.50
3	60	43.50
4	80	63.91
5	100	71.20

Table 7: Showing Inhibitory Activity of DCP on Reducing Power

S. No	Concentration (μg/ml)	% scavenging activity
STD (Ascorbic Acid)		
1	100 μg/ml	88.203
2	200 μg/ml	89.733
3	300 μg/ml	89.76
4	400 μg/ml	90.070
5	500 μg/ml	90.48

DCP		
1	100 µg/ml	80.59
2	200 µg/ml	85.02
3	300 µg/ml	87.79
4	400 µg/ml	92.56
5	500 µg/ml	96.44

Table 8*: In-vivo* Anti-oxidant Activity in Colon Tissue for DCP

GROUPS (n=6)	CONTORL Group-I	Only DMH Group-II	DMH+DCP (200 mg/kg) Group-III	DMH+DCP (400 mg/kg) Group-IV
PROTEIN Mg/G Tissue	0.4900 ±0.02309	0.2390 ±0.02610	0.4040 ±0.007024**	0.4117 ±0.01922***
SOD (units/min/mg protein)	5.460 ±0.2272	2.827 ±0.08090	3.380 ±0.02646***	3.930 ±0.09849***
CATALASE (moles of H2O2 /min/mg protein)	41.60 ±0.863	28.46 ±0.7681	24.40 ±0.2797**	28.41 ±0.7664#
GPx Gram/mg protein	257.8 ±6.693	84.40 ±1.080	259.7 ±3.869***	233.5 ±14.17***
GSH Glutathione Gram/mg protein	107.6 ±5.205	79.97 ±4.383	97.11 ±10.26**	106.6 ±12.89**
LPx MDA formed/mg protein	103.2 ±3.215	84.59 ±2.422	60.94 ±1.138***	61.53 ±1.273***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant
Data is expressed as Mean±SEM. (n = 6, animals in each group). Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.

Table 9: *In-Vivo* Anti-oxidant Activity in Colon Tissue for DCP

GROUPS (n=6)	CONTORL Group-I	Only DMH Group-II	DMH+DCP (200 mg/kg) Group-III	DMH+DCP (400 mg/kg) Group-IV
PROTEIN Mg/G Tissue	06433 ±0.02906	0.2700 ±0.02646	0.5610 ±0.01950***	0.5633 ±0.02186***
SOD (units/min/mg protein)	7.717 ±0.1989	2.437 ±0.0581	4.043 ±0.06642**	4.183 ±0.1126***
CATALASE (moles of H2O2 /min/mg protein)	51.01 ±1.324	24.15 ±0.5355	32.67 ±0.2805**	37.17 ±0.2443**
GPx Gram/mg protein	207.6 ±5.485	73.74 ±2.681	189.9 ±12.42***	174.3 ±8.486***
GSH Glutathione Gram/mg protein	130.1 ±9.655	81.27 ±4.816	99.53 ±1.048#	118.8 ±3.627**
LPx MDA formed/mg protein	92.15 ±1.791	68.14 ±2.752	85.17 ±1.530#	90.14 ±1.609***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant. Data is expressed as Mean±SEM. (n = 6, animals in each group). Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.

Table 10: Estimation of Fructose-1-6-Diphosphatase for DCP

GROUPS (n=6)	CONTORL	Only DMH	DMH+DCP (200 mg/kg)	DMH+DCP (400 mg/kg)
Fructose-1-6-Diphosphatase	86.83 ±2.850	46.67 ±2.657	54.90 ±2.139***	66.57 ±1.866**
Glucose-6-Phosphate	81.54 ±0.8634	51.70 ±1.289	52.59 ±0.4795#	66.25 ±1.065***

***P<0.001, **P<0.01, *P<0.05, #-Non Signifiant. Data is expressed as Mean±SEM. (n = 6, animals in each group). Statistical comparison**:** One way ANOVA, followed by Dunnet's comparison was performed.

Fig 1: Invitro Free radical scavenging activity of DCP in various methods.

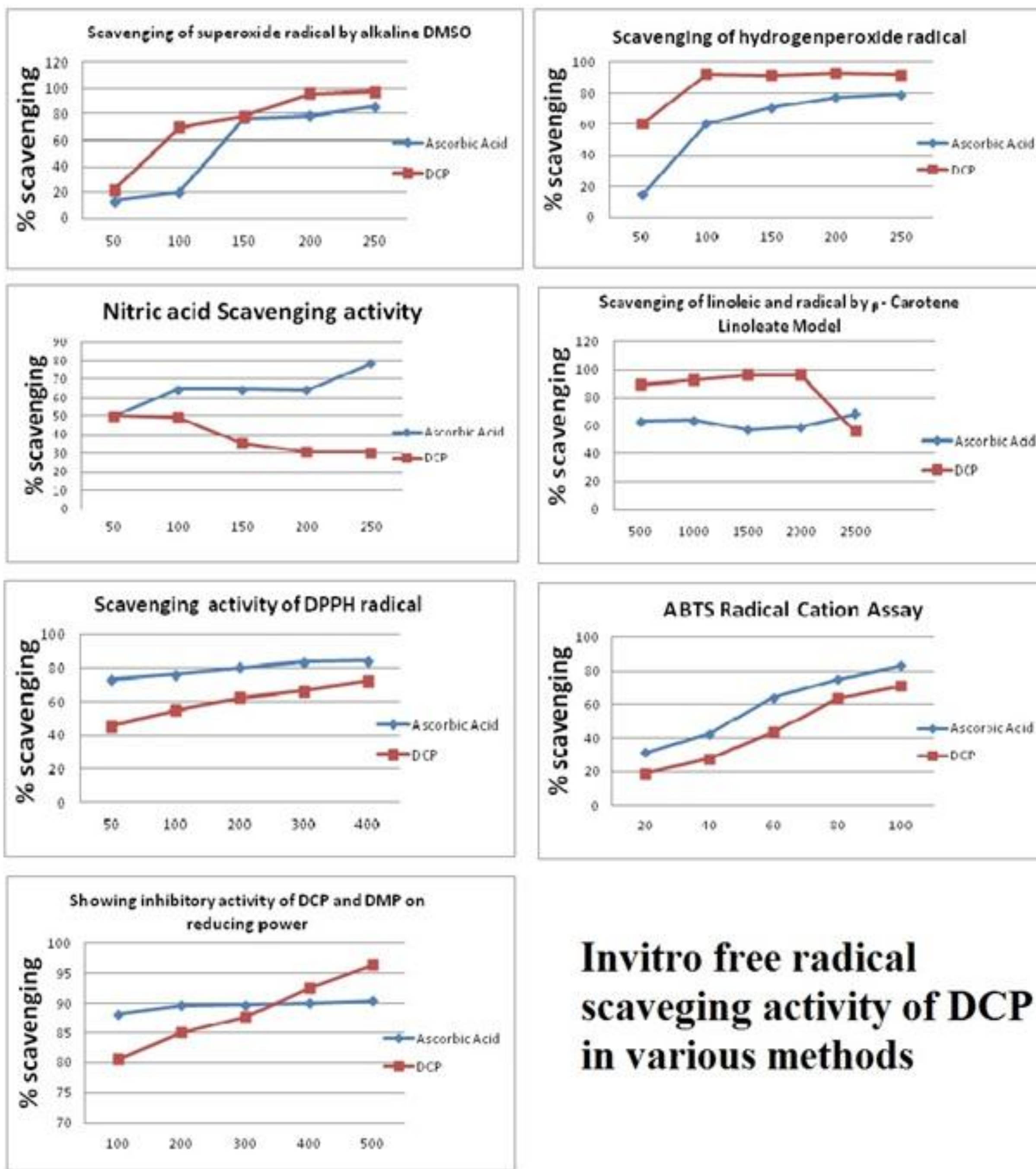


Fig 2: In-Vivo Anti Oxidant Activity in colon Tissue for DCP

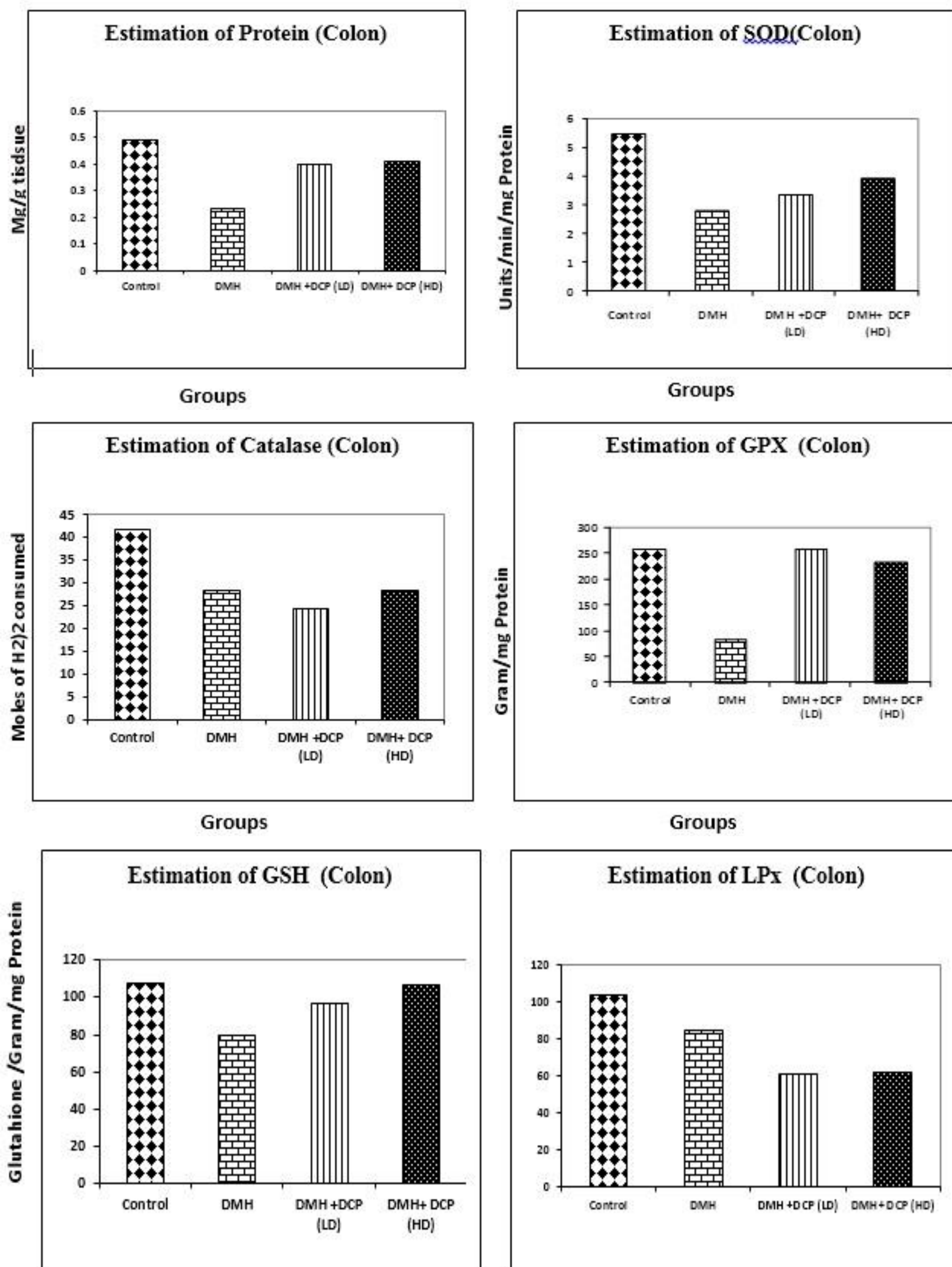
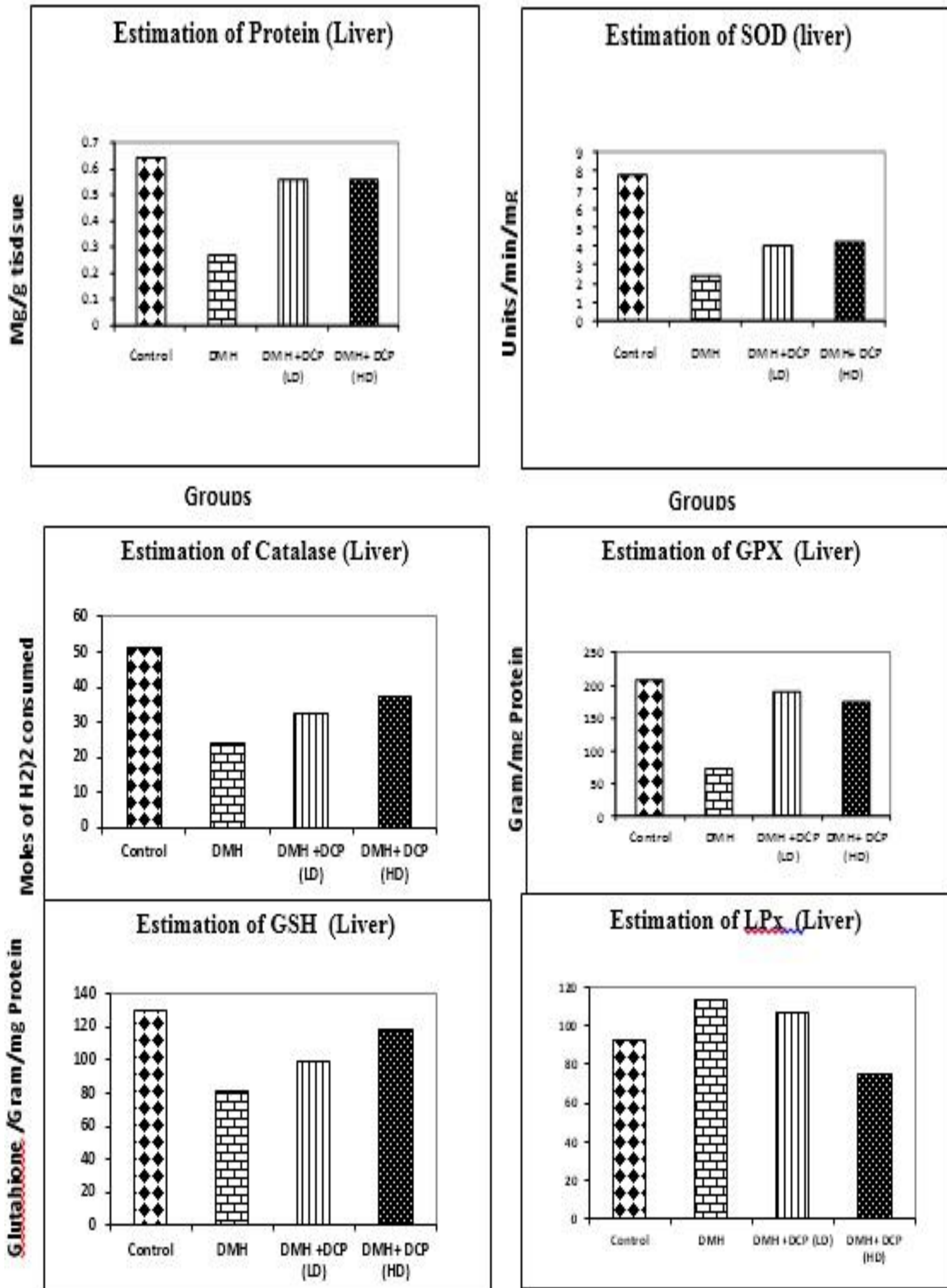


Fig 3: In-Vivo Anti-oxidant Activity in Liver Tissue for DCP



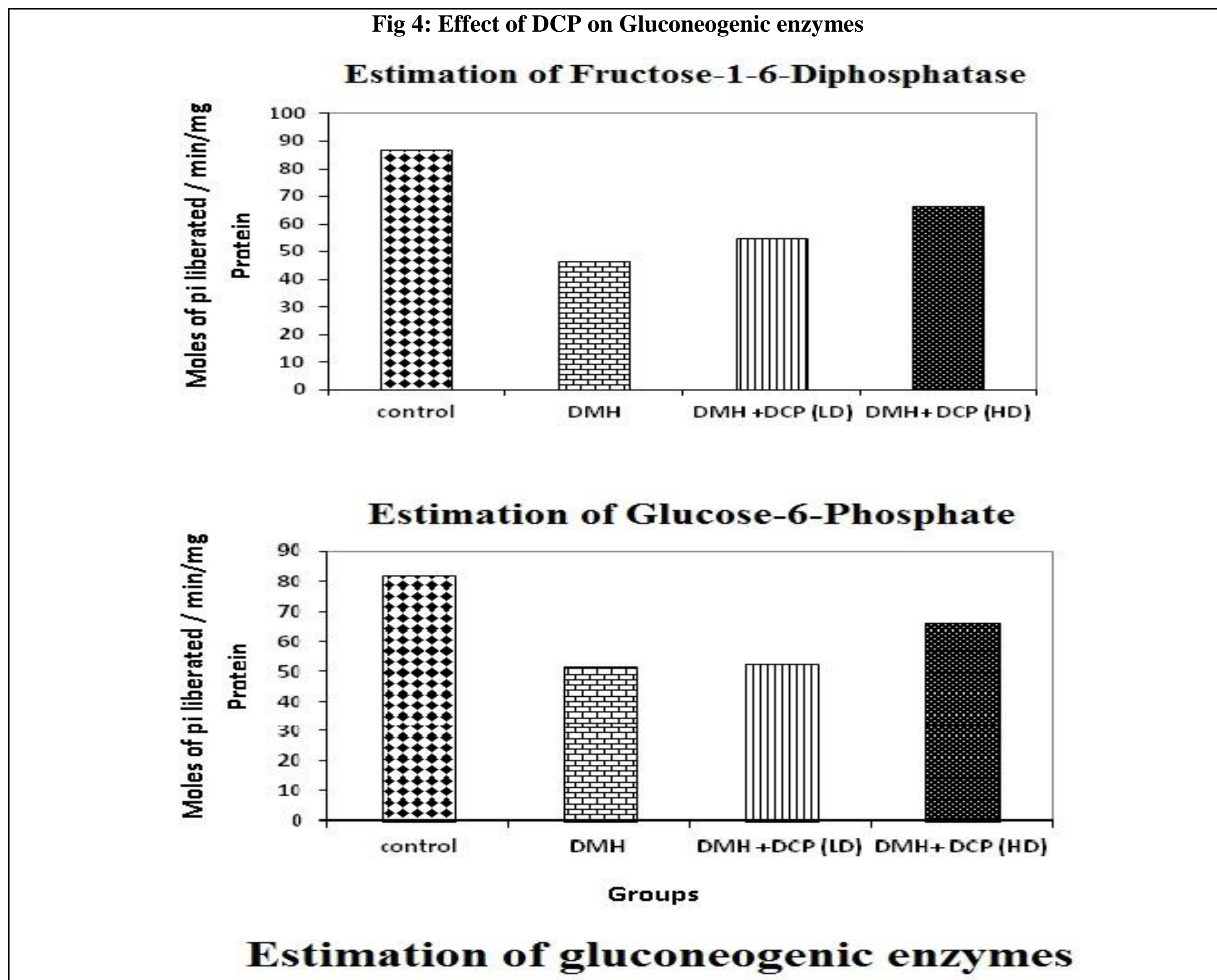


Fig 4: Effect of DCP on Gluconeogenic enzymes

CONCLUSION

DCP was also studied for its *in vitro* antioxidant property by different methods. DCP has a hydrogen donating property, which may be attribute to the good antioxidant activity. The *in vitro* data suggest that the extract is having both the *in vitro* antioxidant and hence, further *in vivo* studies were proceeded with DCP.

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